cytiva

ÄKTA oligopilot plus

Operating Instructions

Original instructions
Translation disc included

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Table of Contents

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1 Introduction

About this chapter

This chapter contains important user information, descriptions of safety notices, regulatory information, intended use of the ÄKTA oligopilot plus systems, and lists of associated documentation.

In this chapter

1.1 About this manual

Purpose of this manual

The *Operating Instructions* provide you with the information needed to install, operate and maintain the product in a safe way.

Scope of this manual

These *Operating Instructions* cover the ÄKTA oligopilot plus 10 and ÄKTA oligopilot plus 100 instruments and UNICORN™ control software. The illustration below shows an ÄKTA oligopilot plus system and the software control system.

Typographical conventions

Software items are identified in the text by ***bold italic*** text.

Hardware items are identified in the text by **bold** text.

In electronic format, references in *italics* are clickable hyperlinks.

1.2 Important user information

Read this before operating the product

All users must read the entire *Operating Instructions* before installing, operating or maintaining the product.

Always keep the *Operating Instructions* at hand when operating the product.

Do not operate the product in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use of the product

ÄKTA oligopilot plus is a fully automated system intended for synthesis of DNA and RNA oligonucleotides. ÄKTA oligopilot plus is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

Prerequisites

In order to operate ÄKTA oligopilot plus in the way it is intended:

- The user must have a general understanding of how a PC and the Microsoft® Windows® operating system works.
- The user must understand the concepts of liquid chromatography.
- The user must read and understand the *Safety instructions* chapter in the *Operating Instructions*.
- ÄKTA oligopilot plus and software must be installed, configured and calibrated according to the *Operating Instructions*.

Definitions

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. See definitions below.

WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

Note: *A note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

1.3 Associated documentation

Introduction

This section describes the user documentation that is delivered with the product, and how to find related literature that can be downloaded or ordered from Cytiva.

System-specific documentation

Documentation	Main contents
ÄKTA oligopilot plus Operating Instructions	Instructions needed to install, operate and maintain ÄKTA oligopilot plus in a safe way.
Declaration of Conformity	Declaration of Conformity for EU and/or other regions.

Software documentation

Together with each system, the following software documentation is supplied providing additional information that applies to ÄKTA oligopilot plus, independent of the specific configuration:

Documentation	Main contents
UNICORN manual package	• The manuals contain detailed instructions on how to administer UNICORN, work with methods, perform runs and evaluate results. • The Online help contains dialog descriptions for UNICORN. The Online help is accessed from the ***Help*** menu.

Component documentation

Documentation for components produced both by Cytiva and by a third-party are, if existent, also included in the document package.

Note: *The ÄKTA component manuals supplied with the ÄKTA oligopilot plus system are mainly written for chromatography applications.*

Data files, application notes and user documentation on the web

To order or download data files, application notes or user documentation, see the instruction below.

Step	Action
1	Go to cytiva.com/oligo.
2	Click ***Instruments***.
3	Click ***ÄKTA oligopilot plus oligonucleotide synthesizer***.
4	Click ***RELATED DOCUMENTS***.
5	Select to download the chosen literature.

2 Safety instructions

About this chapter

This chapter describes safety precautions, labels and symbols that are attached to the equipment. In addition, the chapter describes emergency and recovery procedures, and provides recycling information.

Important

WARNING

Before installing, operating or maintaining the product, all users must read and understand the entire contents of this chapter to become aware of the hazards involved.

In this chapter

2.1 Safety precautions

Introduction

ÄKTA oligopilot plus is powered by mains voltage and handles materials that can be hazardous. Before installing, operating or maintaining the system, you must be aware of the hazards described in this manual.

Follow the instructions to avoid injury to the operator or other personnel, damage to samples or other substances handled by the equipment, to the product, or to other equipment in the area.

The safety precautions in this section are grouped into the following categories:

- General precautions
- Personal protection
- Flammable liquids and explosive environment
- Installing and moving the product
- Operation
- Maintenance

General precautions

WARNING

Do not operate the product in any other way than described in the user documentation.

WARNING

Only properly trained personnel may operate and maintain the product.

WARNING

Do not use any accessories not supplied or recommended by Cytiva.

WARNING

Do not use ÄKTA oligopilot plus if it is not working properly, or if it has suffered any damage, for example:

- damage to the power cord or its plug
- damage caused by dropping the equipment
- damage caused by splashing liquid onto it

Personal protection

WARNING

Always use appropriate Personal Protective Equipment (PPE) during operation and maintenance of this product.

WARNING

Hazardous substances. When using hazardous chemicals, take all suitable protective measures, such as wearing protective clothing, glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the product.

WARNING

High pressure. The product operates under high pressure. Wear protective glasses and other required Personal Protective Equipment (PPE) at all times.

Flammable liquids and explosive environment

WARNING

Fire Hazard. Before starting the system, make sure that there is no leakage.

WARNING

A fume hood or similar ventilation system shall be installed when flammable or noxious substances are used.

WARNING

Explosion hazard: The product uses potentially explosive liquids. Consult local authorities regarding local rules and regulations before installing or operating.

Installing and moving the product

WARNING

The product must be installed and prepared by Cytiva personnel or a third party authorized by Cytiva.

WARNING

Supply voltage. Before connecting the power cord, make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument.

WARNING

Protective ground. The product must always be connected to a grounded power outlet.

WARNING

Power cord. Only use power cords with approved plugs delivered or approved by Cytiva.

WARNING

Do not block the ventilation inlets or outlets on the system.

WARNING

Installing the computer. The computer must be installed and used according to the instructions provided by the manufacturer of the computer.

WARNING

Access to power switch and power cord with plug. Do not block access to the power switch and power cord. The power switch must always be easy to access. The power cord with plug must always be easy to disconnect.

WARNING

If the system is moved, the external capillaries and other tubing may become entangled in nearby objects and be pulled from their connections, thereby causing leakage. In addition, prior to moving the system, make sure that all of the inlets and outlets are sealed, or that the system is completely emptied of solvents. Otherwise, there is a risk of leakage and danger for people and/or surrounding equipment.

WARNING

The Inert gas supply must never exceed 0.5 bar.

WARNING

Disconnect power. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.

WARNING

Before connecting a column, read the instructions for use of the column. To avoid exposing the column to excessive pressure, make sure that the pressure limit is set to the specified maximum pressure for the column.

WARNING

Reagent bottles that are connected to the system inlets must be of high quality since they are pressurized during operation. Immediately replace any reagent bottles showing signs of wear or cracks.

CAUTION

Heavy object. Use suitable lifting equipment when moving the systems. Three people are required to lift the system safely.

NOTICE

Disconnect power. To prevent equipment damage, always disconnect the power from the product before an instrument module is removed or installed, or a cable is connected or disconnected.

NOTICE

Any computer used with the equipment must comply with IEC 60950 or IEC 62368-1 and be installed and used according to the manufacturer's instructions.

Operation

WARNING

Before operation, all process connections and the piping system must be tested for leakage at maximum pressure for continued protection against injury risks due to fluid jets, burst pipes or potentially explosive atmosphere.

WARNING

Before beginning work, a solvent-resistant waste container must be connected to the system.

WARNING

High intensity UV light. This product uses high intensity ultra-violet light. Do not disconnect the optical fibers while the lamp is on.

WARNING

Never place waste containers on top of the system. If they become full and overflow, liquid may penetrate the system causing a short-circuit and risk of fire.

WARNING

Never place solvent containers on top of the valve door. If this is done, containers may fall when the valve door is opened.

WARNING

All solvents are volatile and should be regarded as hazardous. Work in a properly ventilated fume hood.

WARNING

- If reagents or solvents come into contact with skin, wash immediately and generously with water.
- If reagents or solvents come into contact with the eyes, wash thoroughly with water and consult a physician as soon as possible.
- If any reagents or solvents are ingested, consult a physician immediately.

CAUTION

Always follow the manufacturer's recommendation for the reagent preparation and use.

CAUTION

Waste tubes and containers must be secured and sealed to prevent accidental spillage.

CAUTION

Make sure that the waste container is dimensioned for maximum possible volume when the equipment is left unattended.

NOTICE

Running out of Inert gas can seriously affect the flow performance of the instrument and put the run at risk. Check the pressure gauges and the feed tank regularly.

NOTICE

Too low pressure in the bottles can cause cavitation.

NOTICE

The free power outlets on the rear panel of the instrument are only intended for using with ÄKTA accessories. Using the power outlets for any other type of equipment might blow the built-in system fuse.

NOTICE

Water will impair oligonucleotide synthesis.

NOTICE

Tetrahydrofurane (THF) is not compatible with the flow paths in the oligonucleotide synthesizers from Cytiva and should therefore not be used in the systems. Reagents containing THF are usually capping solutions and oxidation.

Maintenance

WARNING

If the door is quickly pulled open to its full extent, the internal capillary tubing may be pulled from their connections causing leakage.

WARNING

Do not open any of the electrical components of the instrument. They contain high voltage circuits which can give a lethal electric chock.

WARNING

Always disconnect the power cord or switch off the power at the outlet before maintaining the system. There is still mains voltage present in the ÄKTA oligopilot plus instrument when it is shut off using the Power Switch.

WARNING

Electrical shock hazard. All repairs should be done by service personnel authorized by Cytiva. Do not open any covers or replace parts unless specifically stated in the user documentation.

WARNING

Disconnect power. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.

WARNING

Do not perform any type of maintenance work while the system is powered electrically or when the piping system is pressurized. Note that the piping system can be pressurized even when the system is closed down.

WARNING

For continued protection from fire hazard, replace only with same type and rating of fuse.

WARNING

Decommissioning. Decontaminate the equipment before decommissioning to make sure that hazardous residues are removed.

WARNING

Release the Inert gas pressure in the bottles before attempting to replace any item on the system.

WARNING

Hazardous chemicals during maintenance. When using hazardous chemicals for system or column cleaning, wash the system with acetonitrile and drain or dry the lines.

WARNING

Use only approved parts. Only spare parts and accessories that are approved or supplied by Cytiva may be used for maintaining or servicing the product.

WARNING

Tipping risk. If heavier instrument components such as the pump are removed from the rack and the door is opened fully, the shift in the centre of gravity of the system may cause the system to tip over.

WARNING

After assembly, the piping system must be tested for leakage at maximum pressure for continued protection against injury risks due to fluid jets, burst pipes or potenitially explosive atmosphere.

WARNING

Before disassembly, check that there is no pressure in the piping system.

CAUTION

Make sure that the piping system is completely leakage free before performing any Cleaning-In-Place (CIP) or Sanitation-In-Place (SIP) on the column.

NOTICE

Cleaning. Keep the exterior of the instrument dry and clean. Wipe regularly with a soft damp tissue and, if necessary, a mild cleaning agent. Let the instrument dry completely before use.

NOTICE

Avoid condensation. If ÄKTA oligopilot plus is kept in a cold room, cold cabinet or similar, keep it switched on in order to avoid condensation.

2.2 Labels

Introduction

This section describes the system label and other safety or regulatory labels that are attached to the product.

System label

The system label is located on the back of the equipment. The system label identifies the equipment and shows electrical data, regulatory compliance, and warning symbols.

Description of symbols on the system label

The following symbols and text may be present on the system label:

Symbol/text	Meaning
	**Warning!** Read the user documentation before using the system. Do not open any covers or replace parts unless specifically stated in the user documentation.
Code no	Instrument assembly number
Serial no	Instrument serial number
Mfg Year	Year (YYYY) and month (MM) of manufacture
Voltage **Frequency** **Max Power** **Fuse**	Electrical requirements: • Voltage (VAC ∼) • Frequency (Hz) • Max. power (VA) • Fuse rating

2.3 Emergency procedures

Introduction

This section describes how to shut down the ÄKTA oligopilot plus instrument in an emergency situation, and the procedure for restarting the ÄKTA oligopilot plus instrument.

The section also describes the result in the event of power failure.

Safety precautions

WARNING

Access to power switch and power cord with plug. Do not block access to the power switch and power cord. The power switch must always be easy to access. The power cord with plug must always be easy to disconnect.

Emergency shutdown

In an emergency situation, stop the run by either pausing the run or switching off the instrument as described in the following table:

Step	Action
1	To pause the run from UNICORN, click the ***Pause*** button.
2	If required, switch off power to the instrument by pressing the **Power** switch to the **0** position. The run is interrupted immediately.

Power failure

The result of a power failure depends on which unit is affected.

Power failure to...	will result in...
ÄKTA oligopilot plus instrument	• The run is interrupted immediately, in an undefined state • The data collected up to the time of the power failure is available in UNICORN
Computer	• The UNICORN computer shuts down in an undefined state • The run continues, but data cannot be saved in UNICORN.

Restart after emergency shutdown or power failure

Follow the steps below to restart the instrument after an emergency shutdown or power failure.

Step	Action
1	Make sure that the condition that caused the emergency shutdown or power failure is corrected.
2	If the instrument was switched off, press the **Power** switch on the instrument. *Result:* The instrument should start and the Instrument display should show **Not connected**.
3	Turn on the computer and monitor.
4	Start UNICORN and connect to the system.

3 System description

About this chapter

This chapter gives an overview of the ÄKTA oligopilot plus system, and a brief description of its function.

In this chapter

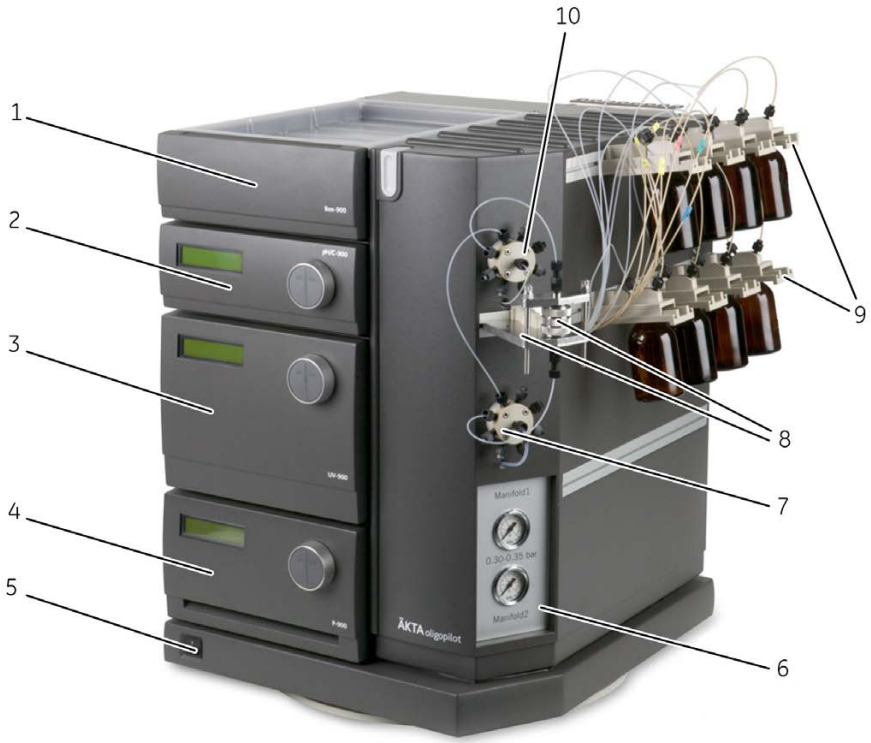
3.1 System overview

Introduction to ÄKTA oligopilot plus

ÄKTA oligopilot plus is a fully automated system intended for synthesis of DNA and RNA oligonucleotides.

Illustration of the instrument

The illustration below shows the main parts of the instrument.

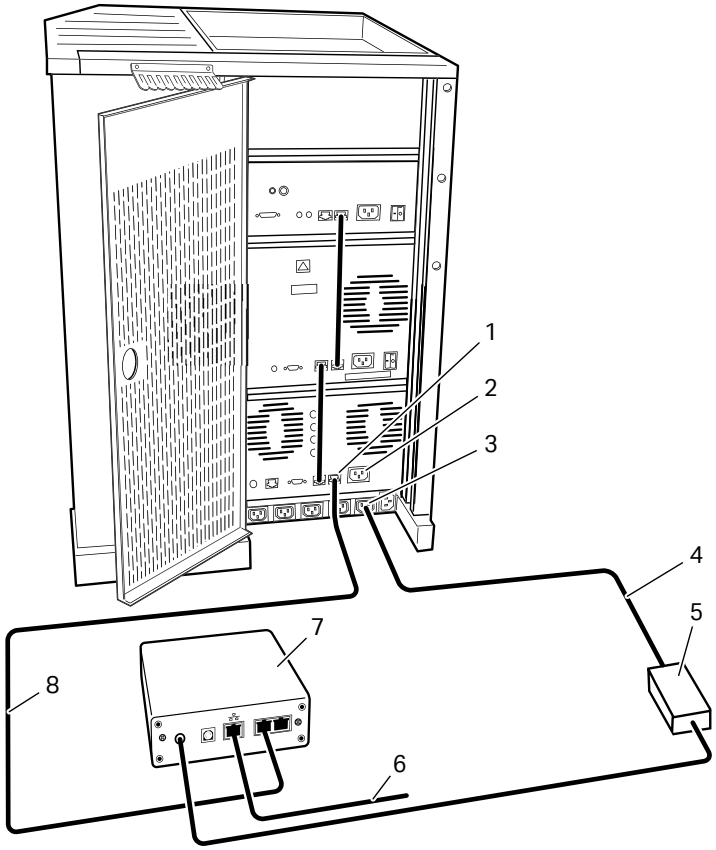


Part	Function
1	Box 900
2	Monitor pH/C-900
3	Monitor UV-900
4	Pump P-900 (P-901 alt. P-903)
5	**Power** switch

Part	Function
6	Pressure gauges
7	Column outlet valve, V7 (IV-908)
8	Column (reactor) and column holder
9	Amidite bottle sliders
10	Column inlet valve, V6 (IV-908)

Electrical and communication connections

The illustration below shows electrical and communication connections.



No.	Description
1	UniNet-1 connector
2	Mains power inlet
3	Mains power outlet
4	Power cord
5	Power supply for CU-950
6	Network cable to computer
7	CU-950
8	UniNet-1 cable

3.2 System components

Introduction

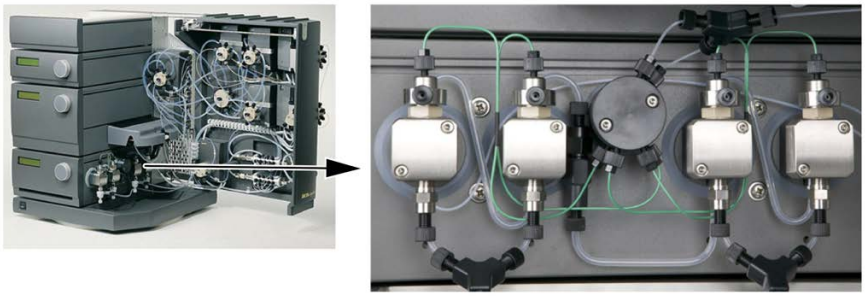
This section describes the different components of ÄKTA oligopilot plus.

In this section

3.2.1 P-900 pumps

The P-900 pumps are high performance laboratory pumps for use where accurate control of the liquid flow is required. The pumps are equipped with two pump modules, each containing two pistons. The pump modules can work independently.

A pressure sensor is connected to one of the pump heads. A Y-connector links the pressure sensor to the other pump head. The maximum flow capacity of the pump installed in ÄKTA oligopilot plus is either 10 mL (model P-903) or 100 mL (model P-901) per pump head.

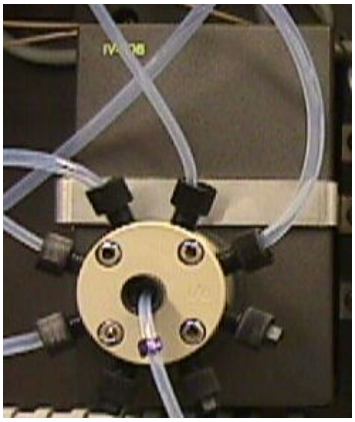


Note: *When used in ÄKTA oligopilot plus 10 or ÄKTA oligopilot plus 100, the pressure limit of the pump is set to 0 to 20 bar.*

3.2.2 Valves

Valve IV-908

Valve IV-908 is a motorized 8-way valve allowing for high flow rates at back pressures up to 20 bar. Valve IV-908 is used for reagent delivery, column selection, column bypass, and waste. All connectors have 5/16" threads.



Valve 8

Valve 8 is a waste selector valve for separating chlorinated hydrocarbons from other waste. Separation is automatically controlled from synthesis methods. The waste selector (see image below) can also be used to separate, for example, the excess of amidites from a coupling.

INV-907H valve

The INV-907H valve is a motorized 7-way valve allowing for high flow rates at back pressures up to 20 bar. The connectors have 5/16" threads.

ÄKTA oligopilot plus contains one INV-907 valve as a re-circulation valve. Position 1 is the flowthrough position, and position 2 is the re-circulation position. Position 3 is not used.

Port	Connection
Port 1	to valve 4
Port 2	stop plug (not used)
Port 3	stop plug (not used)
Port 4	to Waste valve
Port 5	stop plug (not used)
Port 6	to column valve 7
Port 7	to pump B

Valve position 1 (flowthrough)

Port	Connection
Valve 4	Flowthrough to pump B
Column	Flowthrough to waste

Valve position 2 (recirculation)

Port	Connection
Pump B	Recirculation to column

3.2.3 Monitors

pH/Cond-900 monitor

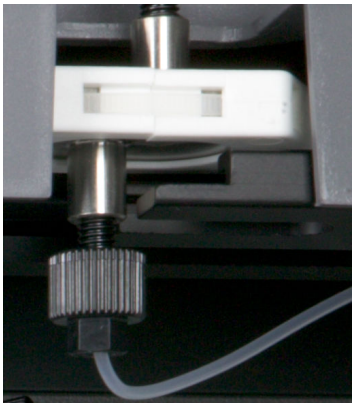
Monitor pH/C-900 is a combined monitor for accurate, on-line monitoring of conductivity and temperature. The pH functionality cannot be used for synthesis applications. The monitor's accurate response, coupled with high precision over a wide measuring range, makes it suitable for any oligosynthesis application.

The monitor pH/C-900 consists of a control unit and a flow cell for conductivity and temperature. The conductivity flow cell (see image below) is installed in the column recirculation loop and can be used to detect and monitor amidite addition during coupling and recirculation steps.

UV-900

Monitor UV-900 is a multiwavelength UV-Vis detector. The detector uses advanced fibre optic technology and is capable of monitoring three wavelengths in the range of 190 to 700 nm simultaneously. The wavelength can be changed using the UNICORN software during a run, either via methods or by manual instruction.

The monitor consists of a main unit, optical fibres and a flow cell (see image below) with an optical path length of 2 mm and an internal volume of 2 µL.



In ÄKTA oligopilot plus applications, wavelengths of 254 nm to 700 nm are utilized which allow the use of low lamp intensity. The low intensity increases the lamp life time. The combination of fibre optics and a unique flow cell design enables a high signal-to-noise ratio with a minimal drift and refractive index effects.

Note: *The detector must be zeroed for each wave length used in a method run. This can be done at any point prior to the wave length being used (e.g., at the beginning of the synthesis run).*

3.2.4 Flow restrictor FR-902

The flow restrictor FR-902 generates a steady back-pressure to prevent air bubbles from being formed after the column in the flow cells. The flow restrictor FR-902 also acts to maintain an even distribution of reagents within the column.

The flow restrictor is set to 2 bar at the factory.

3.2.5 Inert gas supply

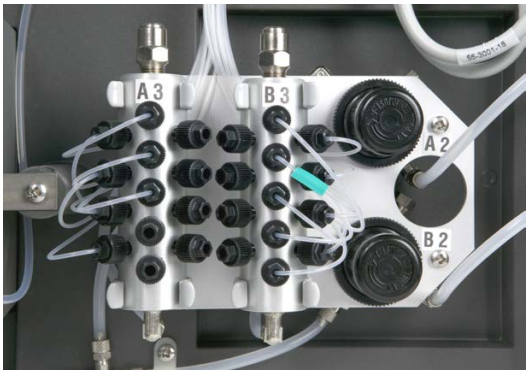
Description

Inert protective gas is used to enable a water-free environment for the reagents.

The gas is delivered from an external gas supply line with a regulator to the gas control of the instrument. The gas is distributed to the reagent bottles via two internal gas regulators and two gas manifolds. The gas control system allows fine adjustment of the inert gas pressure to 0.30 to 0.35 bar, with a gas supply line inlet pressure of 0.45 to 0.50 bar, maximum 0.5 bar.

Note: *If separate gas control for external tanks are used, e.g. large acetonitrile or detritylation supply tanks, make sure to set the pressure to match that on the ÄKTA oligopilot plus. This enables the same gas pressure in all bottles and containers.*

The inert gas is distributed via two manifolds (see below), fitted with gas regulator adjustment knobs. The manifolds are equipped with relief valves set to 0.5 bar.



The activator and thiolation bottles are connected by a Y-connector (tubing marked with green color), and amidite bottles are fed from the right manifold, gauge **Manifold 2**. Acetonitrile, oxidation, capping, thiolation, detritylation reagents etc. are fed from the left manifold, gauge **Manifold 1**. The pressure gauges are shown in the image below.

Inert gas recommendations

CAUTION

Using helium as an inert gas is allowed, but not recommended. The helium will exit any open vessel. This increases the risk of air and moisture entering the bottle, decreasing the effectiveness of the inert gas.

Recommended protective inert gases are high quality, very dry argon and nitrogen. Argon is heavier than air and will form a protective layer, preventing humidity from the air to enter the reagents. Nitrogen is a less expensive alternative and slightly lighter than the air but has the ability to protect the reagents sufficiently.

Argon regulator

There are two argon regulators located on the inside of the door of ÄKTA oligopilot plus and two gauges located on the corner of the system. The regulator should be set to 0.3 bar and will create a low over-pressure in the reagent bottles in order to keep an air/water free environment. The argon pressure also creates a positive pressure on the inlets, which will assist the pumps in creating a steady flow rate.

It is important to have an equal pressure on all reagent bottles so that the pumps will deliver the flow rate set in the method. If the pressure is too low in the bottles it may cause cavitation. If the pressure is too high it may cause a higher flow rate than programmed in the synthesis method.

3.2.6 Accessories

Bottle caps and connectors

Special bottle caps provide a tight seal and prevent the reagents from humidity. The bottle caps have three separate tubing connectors for delivery of solvents delivery and one for inert gas.

The bottle caps are designed to fit standard Schott, Duran and Pyrex bottles with GL-45 threading.

CAUTION

Remove any pouring ring from the bottle before fitting the bottle cap, or the sealing will be damaged, risking gas leakage.

Connectors and tubing

Solvent-resistant fluorinated plastic tubing is used in ÄKTA oligopilot plus for inert gas delivery and reagent/solvent delivery. The tubing is optimized in length and diameter. To connect new bottle caps, the following items are required:

- **Solvent tubing**
  - PEEK or FEP tubing
  - three appropriate tubing connectors
  - ferrules for the tubing connectors, to connect to amidite valve or reagent inlet panel and inlet filter
- **Inert gas tubing**
  - ETFE or FEP tubing
  - two appropriate tubing connectors
  - ferrules for the tubing connectors, to connect to the amidite gas manifold or reagent inlet panel

Tubing specification

Tubing connector marking	Tubing type	Outer diameter of tubing
1A	Solvent tubing	3/16"
1B	Solvent tubing	3/16"
2	Solvent or amidite tubing	1/16" or 1/8"
G	Inert gas tubing	1/16" or 1/8"

Note: *Unused connections must be fitted with a 3/16" stop plug and tightened sufficiently to prevent inert gas from leaking out of the bottle.*

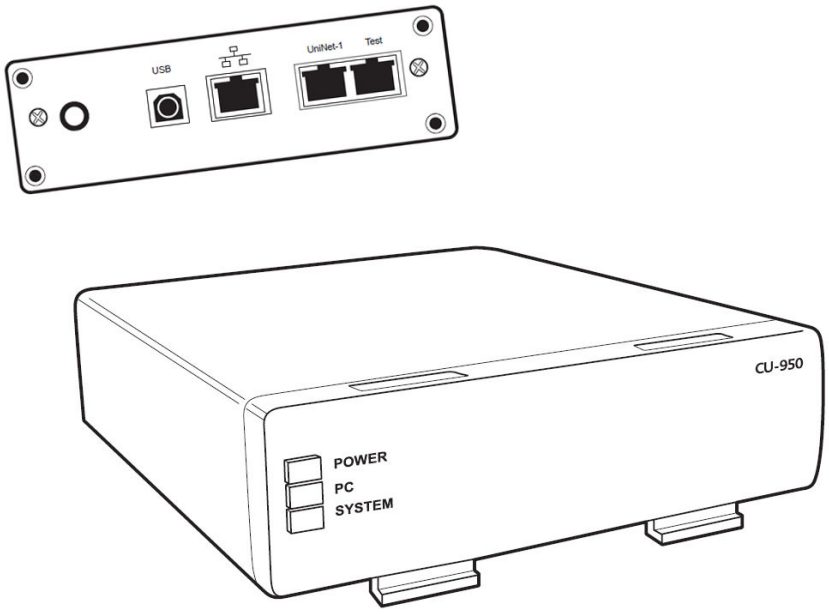
For full tubing specifications, see *Appendix A Tubing, on page 175*.

For ordering information on replacement tubing, see *Section 11.5 Ordering information, on page 172*.

CU-950 controller

The CU-950 is an external controller unit which is connected to the computer and the ÄKTA oligopilot plus system.

The illustration below shows the front and rear panels of the CU-950 unit.



Amidite bottle slider

The standard ÄKTA oligopilot plus is provided with eight amidite bottle sliders. Each slider can hold a standard 100 mL bottle. Four additional sliders can be included.

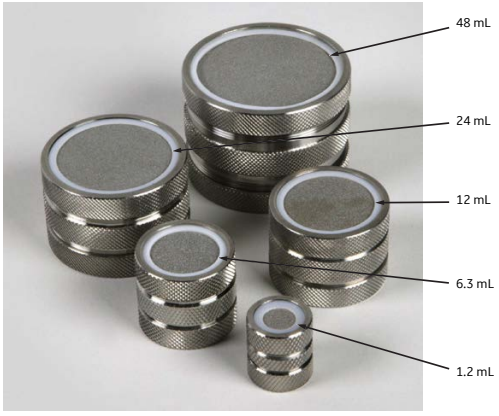
See *Connecting the amidite bottle slider, on page 80*

3.2.7 Columns and reactors

Column reactor

The column reactor holds the solid support on which the synthesis is performed. After completed synthesis, the column is removed from ÄKTA oligopilot plus and the support is processed separately to recover the oligonucleotide product.

The column reactors are made of stainless steel, with a frit pore size of 20 µm.



Column reactor data

Column reactor	Exact volume (mL)	Inner diameter (mm)	Height (mm)
1.2 mL	1.18	10	15
6.3 mL	6.28	20	20
12 mL	12.02	27	21
24 mL	24.05	35	25
48 mL	48.05	44	31.6

Column reactor holders

The column reactor is fitted in a column holder, suitable for column reactors with volumes from 1.2 up to 48 mL. The column holder is supplied together only with the ÄKTA oligopilot plus 100, and is a required accessory for using 1.2 mL columns with ÄKTA oligopilot plus 10.

FineLINE 35 oligo column

The FineLINE™ 35 oligo column is suited for column volumes from 10 to 100 mL. The scale range depends on the loading of the support. The filter is made of stainless steel, with a frit pore size of 20 µm. The column has a hydraulically adjustable adapter.

The column is intended for pilot scale method development or small-scale production of oligonucleotides using Primer Support™ 5G.

For more information, see *FineLINE 35 oligo column Operating Instructions*, product code 28964957.

Adjustable oligo column

The Adjustable oligo column is suited for column volumes of 30 to 200 mL. The filter is made of titanium, with a frit pore size of 20 µm.

The column is intended for pilot scale method development or small-scale production of oligonucleotides using Primer Support 5G.

For more information, see *Adjustable oligo column Operating Instructions*, product code 28967444.

3.3 Control software

UNICORN control software

ÄKTA oligopilot plus is controlled by UNICORN software. UNICORN is a complete package for control and supervision of chromatography/oligo systems. It consists of control software and a controller card or unit for interfacing the controlling PC to the chromatography liquid handling module. The software is operated using Microsoft Windows operating system.

UNICORN is supplied with a number of ready-made method templates that provide easy creation of methods for synthesis.

For more information about the UNICORN control system, see the UNICORN user manuals supplied.

4 Installation

About this chapter

This chapter provides required information to enable users and service personnel to unpack, install, move and transport the ÄKTA oligopilot plus system.

In this chapter

4.1 Site requirements

Environmental requirements

Parameter	Requirement
Allowed location	Indoor use
Placement	Stable laboratory bench min. 200 x 80 cm
Ambient temperature	4°C to 40°C
Humidity	20% to 95%, non-condensing
Atmospheric pressure	840 to 1060 mbar (84 to 106 kPa)
Altitude, operation	Maximum 2000 m
Pollution degree of the intended environment	2

Make sure the working area is well-ventilated, preferably with a fume hood, since organic solvents are used with the instrument.

Electrical power requirements

Parameter	Requirement
Supply voltage	100-120/220-240 V AC
Frequency	50 to 60 Hz
Transient overvoltages	Overvoltage category II

4.2 Transport

Introduction

The equipment weighs 63 kg and requires at least three people to lift and move it unless a suitable lifting device is used.

The equipment can be transported on a trolley capable of supporting at least 80 kg.

NOTICE

Lift the instrument in the upright position. Do not use the front panel bar as a lifting handle.

Before moving the system

Follow the steps below to prepare the system for transport.

Step	Action
1	Start the shutdown method template.
2	Rinse the system with acetonitrile.
3	Empty the system using inert gas.
4	Disconnect all cables and tubing connected to peripheral components and liquid containers.
5	Close the door completely.

Moving the system

CAUTION

Heavy object. Use suitable lifting equipment when moving the systems. Three people are required to lift the system safely.

Grasp the system firmly by placing the fingers in the gap between the swivel platform and the base of the main unit and lift.

4.3 Unpacking

Check for damage

Check the equipment for damage before starting assembly and installation as follows.

- Check that there are no loose parts in the transport crate.
- Check that all the parts are either mounted on the system or located in the accessory kit box.

If any damage is found, document the damage, and contact your local Cytiva representative.

Unpack the system

Remove straps and packing material, then stand the equipment upright on its swivel foot before starting installation.

4.4 Assembly

Assembly before use

The following parts must be connected to the ÄKTA oligopilot plus instrument before it can be used:

- Waste container
- CU-950 Control unit connected between the computer and the ÄKTA oligopilot plus system.
- Amidite bottle sliders
- Bottle caps for reagent bottles

4.5 Connections

Communication

Connect the network, signal cables and computer according to the electrical drawings in *Electrical and communication connections, on page 30*

Make sure that UNICORN control software is installed on the computer.

Installing Controller unit CU-950

Hang the CU-950 on the left side of the system by inserting the hooks on the front of CU-950 into the groove on the side of the UV-900 and rotating into position.

Connect according to diagram in *Electrical and communication connections, on page 30*

Electrical power

Connect the power cord to a grounded power outlet as specified in *Section 4.1 Site requirements, on page 49*.

5 Installation verification and reinstallation

About this chapter

This chapter contains information on how to verify the installation.

In this chapter

5.1 Installation test

Purpose of the installation test

The installation test is designed as a preliminary test of the function of ÄKTA oligopilot plus, but this test can also be used to periodically test the solvent delivery system and UV monitor in ÄKTA oligopilot plus.

Note: *The UV monitor is tested by measuring the UV response at 254 nm, using a solution of 0.2 % toluene or 1 % acetone dissolved in acetonitrile.*

WARNING

Always use appropriate Personal Protective Equipment (PPE) during operation and maintenance of this product.

Installation test overview

The installation test is performed in 7 steps. Each step is described in detail in the following text.

WARNING

Fire Hazard. Before starting the system, make sure that there is no leakage.

Step	Action
1	System setup
2	Prepare the system for an installation test
3	Purge the P-900 pump
4	Prime the piston seal rinsing system of the P-900 pump
5	Test the pressure stability
6	Run the installation test method
7	Evaluate the results of the installation test

System setup

Follow the steps below to start the system.

Step	Action
1	Switch on the system unit using the **Power** switch located to the left on the base platform.
2	Switch on the computer, the display and the printer according to the instructions in the manufacturer manuals.
3	Log on to Windows.
4	When the Windows desktop appears, start UNICORN by double-clicking on the UNICORN icon.
5	Select user ***default*** and enter ***default*** as the password. Click ***OK***.
6	Click the system control icon on the task bar.

Prepare the system for an installation test

Follow the steps below to prepare the system for an installation test.

Step	Action
1	Open the main valve on the inert gas tank and adjust the out pressure to 0.5 bar (7 psi) on the inert gas regulator.
2	***Note:*** *Perform the following steps quickly in order to minimize gas waste.* With no bottles connected to the instrument, adjust the ÄKTA oligopilot plus gas regulator to 0.30-0.35 bar. Gas should now be coming out of the amidite gas lines and reagent gas lines.
3	Connect the bottles with reagents or acetonitrile to the reagent inlet panel as described in *Section 6.5 Connecting reagents and solutions to the inlet panel, on page 78*.
4	Connect the bottles with acetonitrile to the amidite bottle sliders.
5	Connect a bottle with 500 mL of either • 1% acetone in acetonitrile, or • 0.2% toluene in acetonitrile to position **13** (gas inlet) on the reagent inlet panel.

Step	Action
6	Connect the bottle with the 1% acetone or 0.2% toluene, in acetonitrile to position **14** (reagent inlet) of the reagent inlet panel.
7	Check that the pressure of inert gas remains at 0.30-0.35 bar. Adjust as necessary.

Purge the P-900 pump

For instructions see *Section 9.3.2 Purging the P-900 pump, on page 116*.

Priming the piston seal rinsing system of the P-900 pump

For instructions see *Section 9.3.3 Priming the piston seal rinsing system of the P-900 pump, on page 117*.

Test the pressure stability

Run this test to make sure that all air has been evacuated from the pump heads.

Follow the steps below to test the pressure stability.

Step	Action
1	Run pump A at 10 mL/min. Check the pump display to see that the pressure reading is stable (fluctuation < 1.0 bar).
2	Run pump B at 10 mL/min. Check the pump display to see that the pressure reading is stable (fluctuation < 1.0 bar). ***Note:*** *Check that there are no leaks in the pump or the connectors.* • If the pressure is stable, click ***END*** and proceed with the next step. • If the pressure fluctuates excessively, troubleshoot the pump according to the instructions in *Section 10.4 Pump P-900, on page 142*.
3	Make sure that 500 mL of any of the following solutions is connected to position 14 in the reagent inlet panel: • 1% acetone in acetontrile • 0.2% toluene in acetonitrile

Run the installation test method

Follow the steps below to run the installation test method.

Step	Action
1	In UNICORN, select ***File*** →***Printer setup...*** from the main menu bar. Select the desired printer in the list and select ***Landscape***. Confirm the choice by clicking ***OK***.
2	Click the ***Instant Run*** icon in the main menu.
3	Select ***Installation test ÄKTA oligopilot plus 100 / ÄKTA oligopilot plus 10*** in the list of templates.
4	Click ***Run***.
5	Click ***Next*** twice in the ***Method*** window.
6	Click ***Start*** to run the installation test method. The method runs for approximately 20 minutes.

Hiding information in the *Curves* window

Follow the steps below to hide information in the ***Curves*** window.

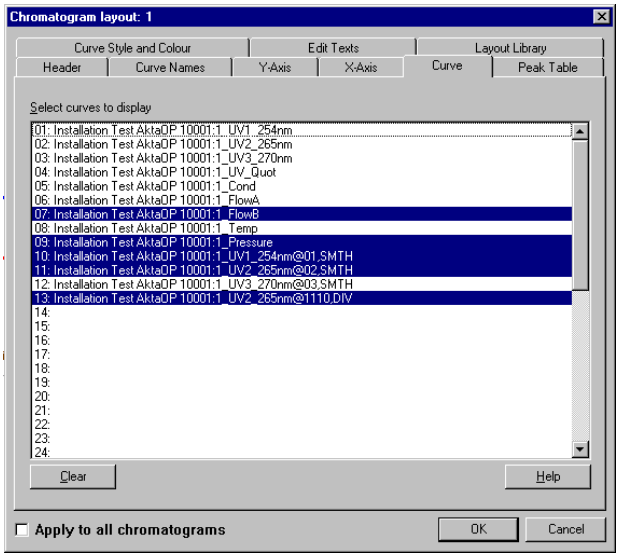
Step	Action
1	Right-click in the ***Curves*** window and select ***Properties*** in the context menu.
2	Click the ***Curves*** tab.
3	Select the following curves to be displayed: • ***UV1_254nm*** • ***Flow B*** • ***Pressure***
4	Deselect all other highlighted curves. The curves may now be monitored on the screen as the test progresses.

Evaluate the results of the installation test

Follow the steps below to bring up the curves from the installation test.

Step	Action
1	Click on the main menu icon on the Windows taskbar.

Step	Action
2	Click on in the results panel.
3	Double-click the ***Installation test ÄKTA oligopilot plus 100 / ÄKTA oligopilot plus 10*** icon to open the result file.
4	Maximise the result window by clicking in the upper right corner.
5	Right-click the ***Curves*** window and select ***Properties***.
6	Select the curves to display as in the following image.



7	Click ***OK***.

Check the pressure curve

Follow the steps below to check the pressure curve.

Step	Action
1	Select the pressure curve and deselect all other curves.
2	Double-click the ***XY*** icon in the upper left corner. The bar reading is displayed.
3	Move the cursor and read the values after 4 minutes. The pressure fluctuation should be larger than 1.0 bar.

Obtain and print the response percentage

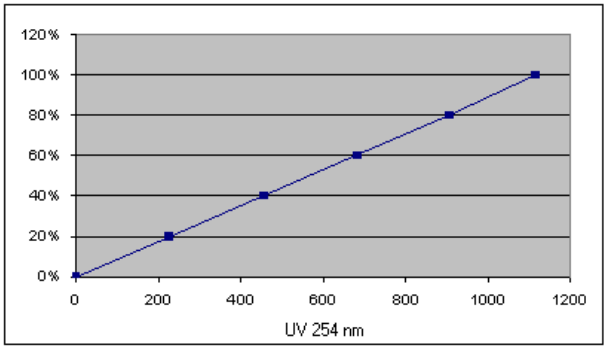
Follow the steps below to obtain and print the response percentage.

Step **Action**

1 Calculate the response percentage using the following table, which contains typical UV values.

Percent B-pump flow	UV 254 nm	Calculation	Percentage
100	1117	1117/1117	**100%**
80	905	905/1117	**81%**
60	680	680/1117	**61%**
40	457	457/1117	**41%**
20	225	225/1117	**20%**
0	0	0/1117	**0%**

2 Plot the stepwise UV values against the 100% to 20% B-flow.



3 Click ***Print*** to obtain a printed report of the results.

5.2 Verification synthesis

Introduction

This section provides background information on the verification synthesis.

Purpose of the verification synthesis

Perform the verification synthesis as a part of system installation or reinstallation and to verify that the ÄKTA oligopilot plus system works after troubleshooting.

The verification synthesis is used to verify that:

- the ÄKTA oligopilot plus system is fully functional
- the synthesis method is working
- the reagents used are usable.

Reagents and time

The reagents and time needed to create a Test13 sequence are described in *Section 6.3 Preparing for a verification synthesis, on page 73*.

5.3 Reinstalling the system

Introduction

This chapter gives instructions on how to reinstall the system.

CAUTION

Heavy object. Use suitable lifting equipment when moving the systems. Three people are required to lift the system safely.

Site preparations

- Create a clean and dry working area of 200 × 80 cm that allows easy access.
- Check that there is a wall outlet with 100-120/220-240 V AC, 50-60 Hz nearby.
- Make sure the working area is well-ventilated, preferably with a fume hood, since organic solvents are used with the instrument.

Reinstallation overview

Reinstallation is performed in the following steps.

Step	Action
1	Connect the inert gas supply.
2	Connect the column and the waste tubing.
3	Connect the reagent bottles.
4	Connect filters to the tubing.

Connecting the inert gas supply

Follow the steps below to connect the inert gas supply.

Step	Action
1	Connect an inert gas regulator, with ¼" hose barb, to an inert gas cylinder. ***Note:*** *Inert gas supply must not exceed 0.5 bar (7 Psi).*
2	Connect the gas inlet line (product code 80208148) to the ¼" hose barb, and connect the other end (quick connect) to ÄKTA oligopilot plus.

Connecting the column and the waste tubing

Follow the steps below to connect the column and the waste tubing.

Step	Action
1	Install the column holder and the column reactor end pieces.
2	Connect two connectors (5/16" female/M6 male, product code 18112776), one at the top and one at the bottom column reactor piece.
3	Cut two pieces of 25-35 cm long ETFE/PEEK tubing (1.0 mm I.D., 1/16" O.D., 18111583).
4	Connect one tubing piece to valve 6 pos. 2 and to the top column reactor piece. Use 1/16" tubing connectors (18112707) and 1/16" ferrules (18112706).
5	Connect the other tubing piece to valve 7 pos. 2 and to the bottom column reactor piece.Use 1/16" tubing connectors (18112707) and 1/16" ferrules (18112706).
6	Connect the waste tubing (FEP, 2.9 mm I.D., 3/16" O.D., 18111247) from valve 8 pos. 1 and valve 8 pos. 2. The length of the tubing is not critical.

Connecting the reagent bottles

For ÄKTA oligopilot plus 10, follow the steps below:

Step	Action
1	Connect tubing for reagent bottles (FEP tubing, 1.6 mm I.D., 1/8 O.D (18112116) and tubing for gas (ETFE tubing, 1.0 mm I.D., 1/16 O.D., 18114238). • For 1/8 O.D. tubing, use 1/8 tubing connectors (18112118) and 3/16 ferrules (18112118). • For 1/16 tubing, use 1/16 tubing connectors (18112707) and 1/16 ferrules (18112706).
2	Connect the tubing to the reagent inlet panel (see *Section 6.5 Connecting reagents and solutions to the inlet panel, on page 78*) and to a bottle cap (complete 4 x 5/16, 18113701). The length of the tubing is not critical.

For ÄKTA oligopilot plus 100, follow the steps below:

Step	Action
1	Connect tubing for reagent bottles (FEP tubing, 2.9 mm I.D., 3/16" O.D., 18111247) and tubing for gas (ETFE tubing, 1.0 mm I.D., 1/16" O.D., 18114238). • For 3/16" O.D. tubing, use 3/16" tubing connectors (18111249) and 3/16" ferrules (18111248). • For 1/16" tubing, use 1/16" tubing connectors (18112707) and 1/16" ferrules (18112706).
2	Connect the tubing to the reagent inlet panel as described in *Section 6.5 Connecting reagents and solutions to the inlet panel, on page 78* and to a bottle cap (complete 4 x 5/16", 18113701). The length of the tubing is not critical.

Connecting filters to the tubing

Attach a filter (or "frit") to the solvent supply tubing of every bottle.

6 Pre-synthesis procedure

About this chapter

This chapter describes the standard preparation required before performing a synthesis with ÄKTA oligopilot plus.

In this chapter

6.1 Required items

Reagents and solvents

The tables below list the reagents and solvents needed for a normal DNA oligonucleotide synthesis.

NOTICE

Tetrahydrofurane (THF) is not compatible with the flow paths in the oligonucleotide synthesizers from Cytiva and should therefore not be used in the systems. Reagents containing THF are usually capping solutions and oxidation.

Note: *For best results, use only reagents recommended by Cytiva.*

DNA (standard) amidites[1]	Notes
dA-amidite	Dissolved in acetonitrile
dC-amidite	
dG-amidite	
T-amidite	
Primer Support 5G	One support for each 3' nucleotide (A, G, C, T)[2]

[1] Virtually any other amidites can be used as well.
[2] Support with other start bases are also available.

Reagents	Notes
Detitrylation solution	3% dichloroacetic acid in toluene
Acetonitrile (ACN)	dry, < 30 ppm water
Activator	0.3 M BTT in acetonitrile • For DNA synthesis, 0.25 M is sufficient
Capping A	N-methylimidazole 20% (v/v) in ACN
Capping B1	Acetic anhydride 40% (v/v) in ACN
Capping B2	Sym Collidine 60% (v/v) in ACN
Oxidation reagents	Iodine 50 mM, in 10% water/pyridine

Reagents	Notes
Thiolation reagents	Examples: • Phenylacetyl disulfide (PADS) • 3-ethoxy-1,2,3-dithiazoline-5-one (Edith)

Molecular sieves

Cytiva recommends using 3 Å molecular sieves in the amidite, activator and acetonitrile bottles. This minimises the risk of water contamination, and extends the stability time the reagents and amidites can be connected to the system. The following 3 Å sieves are recommended:

- Molecular sieves 3 Å, rods
- Molecular sieves 3 Å, 2 mm beads
- Molecular sieves 3 Å, 2 mm beads

Note: *Do not reuse molecular sieves.*

Molecular sieves must be activated before use (if not preactivated at delivery) by heating to 150°C to 200°C overnight under high vacuum. Use a vacuum oven or a vacuum flask and heating mantle. Allow the sieves to cool to room temperature under vacuum, then transfer them to a dry jar with a tight-fitting lid. Keep the jar in a closed desiccator containing a drying agent.

Filters

Cytiva recommends using bottle/vial filters in the reagent and solvent bottles. Change the filters whenever the bottle is changed. Bottle/vial filters are supplied in packs of 500 (product code 18102985) and fit the O.D. 1/16" tubing. When using reagent bottles, reagent bottle filters (product code 18111315), which fit the O.D. 3/16" tubing, should be used.

Note: *Do not use the filter part in detrit bottles with chlorinated solvents, or the filter swells and falls out.*

Note: *Make sure to keep the tubing at the bottom of the bottle.*

Primer Support 5G

ÄKTA oligopilot plus methods and columns are optimized using Primer Support 5G as the solid support for synthesis. The polystyrene bead is supplied by Cytiva and primed with a traditional nucleoside base (DNA or RNA) or UnyLinker™ serving as a universal starting linker suitable for DNA and 2' OMe synthesis. For customized alternatives, contact your local Cytiva representative.

Cleavage and deprotection reagents

The support is cleaved from the oligonucleotide after synthesis using concentrated (25% to 30%) ammonium hydroxide.

6.2 Preparing the solutions

Introduction

This section describes the preparation of reagents and solvents for use with ÄKTA oligopilot plus.

Safety precautions

WARNING

All solvents are volatile and should be regarded as hazardous. Work in a properly ventilated fume hood.

WARNING

Hazardous substances. When using hazardous chemicals, take all suitable protective measures, such as wearing protective clothing, glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the product.

WARNING

- If reagents or solvents come into contact with skin, wash immediately and generously with water.
- If reagents or solvents come into contact with the eyes, wash thoroughly with water and consult a physician as soon as possible.
- If any reagents or solvents are ingested, consult a physician immediately.

CAUTION

Always follow the manufacturer's recommendation for the reagent preparation and use.

Precautionary measures: anhydrous conditions

Moisture can significantly decrease the synthesis result. For optimal results, take the following precautions:

- Work quickly with open bottles to avoid allowing any moisture into the reagents and solvents. Traces of water in the reagents and solvents significantly decrease the synthesis efficiency.
- Always use 3 Å molecular sieves in the amidite, activator and acetonitrile bottles.
- Make sure that the inert gas supply is dry and free from carbon dioxide.

Reagents

The following table describes the required reagents. The amount of reagents used per cycle at different synthesis scales depends the degree of substitution and the weight of the support in the column.

Tip: *Do not prepare more reagent than necessary. Many of the reagents have a limited shelf life. Refer to the supplier of each reagent.*

Reagent	Description
Acetonitrile	Make sure that the acetonitrile is anhydrous (less than 30 ppm of water).
Amidites	Amidites are white to off-white solids sold in standard vials, and should be dissolved in anhydrous acetonitrile before the synthesis. See *Attaching the amidite bottles, on page 72*
Activator	Cytiva recommends using benzyl thio tetrazole (BTT) as an activator, with a concentration of 0.3 M. For DNA synthesis, a concentration of 0.25 M is sufficient. Make sure that the activator is fully dissolved, then add 3 Å molecular sieves (enough to cover the filters) before connecting the bottle to the line marked **16** on the reagent inlet panel of ÄKTA oligopilot plus, see *Section 6.5 Connecting reagents and solutions to the inlet panel, on page 78*. ***Note:*** • *Make sure the activator is completely dissolved. Dissolving the activator takes longer as saturation is approached.* • *Do not add molecular sieves until the activator is completely dissolved.* • *The activator may crystallize in the solution at temperatures below 18°C. In this happens, shake the activator solution vigorously at ambient temperature until it clears.*
Oxidizing reagent	The oxidizing reagent is 50 mM iodine (12.7 g I_2 to 1 L solution) in water/pyridine 1:9 v/v).
Capping reagent A	Capping reagent A is 20% N-methylimidazole (v/v) in acetonitrile. This reagent is available as a ready-made solution.

Reagent	Description
Capping reagent B	Capping reagent B is 20% acetic anhydride in 30% sym-collidine, or lutidine, and 50% acetonitrile. This reagent is available as two ready-made solutions to be mixed immediately before use.
Thiolation reagent	Several compositions of thiolation reagents are commonly used, some of them are proprietary and are protected by patents. Make sure that the correct method parameters are used for the chosen reagent. Thiolation (Beaucage) reagent is used at 0.5 M or 10% (w/v) in dry acetonitrile not dried over 3 Å molecular sieves. It is supplied as a solid for preparing the solution. Thiolating reagents such as phenylacetyl disulfide (PADS) dissolved in piccoline/acetonitrile 1:1 (patent protected) can also be used. Another thiolation reagent alternative is Edith. Recommended parameters are the following: • concentration of 0.15 M • volume equal to 0.5 column volumes • contact time of 1 minute.

For connecting reagents and solutions to the inlet panel, see *Section 6.5 Connecting reagents and solutions to the inlet panel, on page 78*.

Detritylation solution

Detritylation solution is 3% v/v dichloroacetic acid (DCA) in toluene or dichloromethane. The solution is stable indefinitely. DCA Toluene can be used with ÄKTA oligopilot plus 10 or ÄKTA oligopilot plus 100. DCA/DCM should only be used with ÄKTA oligopilot plus 100 because DCA/DCM causes de-gasing which may prevent the ÄKTA oligopilot plus 10 pump from performing properly.

Note: *Upon removal of the DMTr group, toluene based solutions generate by-products with only negligible conductivity. Therefore, it is not possible to use conductivity to measure the detritylation efficiency of toluene based solutions.*

Inert gas supply

WARNING

The Inert gas supply must never exceed 0.5 bar.

Note: *Make sure the inert gas supply is free from water and carbon dioxide, both of which can seriously reduce the synthesis efficiency.*

Note: *When changing the inert gas cylinder, make sure there is no condensation in the regulator or gas tubing. Flush the regulator with inert gas from the new cylinder before connecting to ÄKTA oligopilot plus.*

Attaching the amidite bottles

The amidite vials are attached to the instrument either using a simple sliding seal attachment or directly from the bottles using the standard GL-45 bottle cap and tubing assembly. When using the sliding seal attachment, make sure that the vial is properly seated on the sealing gasket and that the gasket is not cracked or worn.

The vial positions on the instrument should be labelled as follows:

A, C, G, T, A*, C*, G*, T*, X, Y, Z, Q

These letters are used to define the oligonucleotide sequence in the sequence editor, and serve as addresses for the amidite vials during synthesis. It is therefore important that amidites are placed in their correct positions on the instrument.

The last bases in the sequence (X, Y, Z, and Q) are reserved for special or customer-designated assignments.

Note:

- *Avoid humidity from entering amidite bottles. Work quickly while the bottle is open.*
- *Before attaching the vial to the instrument, add 3 Å molecular sieves to a depth of approximately 5 mm.*

Note: *To add more amidite bottles, additional amidite bottle sliders are required. For installation instructions, see Section 6.6 Connecting additional amidities, on page 79.*

6.3 Preparing for a verification synthesis

Notes and tips on reagent preparation

When preparing reagents for a synthesis run, consider the following tips.

- Waste should be collected in a closed but ventilated container. When selecting the size of the waste container, select an adequately large container to avoid overfilling the waste. For a verification run a 5 L waste container is enough.
- Prepare more reagents than the minimum requirements.

Reagents preparation for verification of ÄKTA oligopilot plus 10

Normal reagent consumption during a Test13 sequence, with ÄKTA oligopilot plus 10 is indicated in the table below.

The specified volume in the table is enough for column washing, purging and final detritylation. The recommended volume is enough for at least 2 syntheses with respect to the amidites.

Reagent	Required volume (mL)	Recommended volume (mL)
Amidite A, 0.1 M	1.85	10
Amidite C, 0.1 M	1.85	10
Amidite G, 0.1 M	1.85	10
Amidite T, 0.1 M	1.85	10
Acetonitrile	900	1200
Detritylation reagent (must be toluene based)	< 250	500
Capping reagent A	23	100
Capping reagent B	23	100
Oxidation reagent	23	100
Activator (0.3 M BTT)	17	100
DEA (20% in ACN)	40	100

Synthesis specification for ÄKTA oligopilot plus 10

- The specified volumes are appropriate for a 1.2 mL column.

- The duration of synthesis is approximately 2.5 hours.
- Waste generated: 1200 mL
- Required waste container: 2000 mL

Note: *The acetonitrile must be extremely dry. The maximum water content in the solution should not exceed 30ppm.*

Reagents preparation for verification of ÄKTA oligopilot plus 100

Reagent consumption during a 260 µmol synthesis of a Test13 sequence, Trityl-OFF, in a 6.3 mL column with ÄKTA oligopilot plus 100 is indicated in the table below.

The volumes below are based on using 1.3 g of support, 200 µmol/g and account for column wash, purges and final detritylation. The **Prepare at least** suggestions will allow at least 2 syntheses with respect to amidites.

Reagent	Requirement	Prepare at least
Primer Support 5G	700 mg, 200 µmol/g	1 mmol available
Amidite A, 0.15 M	9 mL	34 mL / 5 g
Amidite C, 0.15 M	9 mL	35 mL/ 5 g
Amidite G, 0.15 M	9 mL	35 mL / 5 g
Amidite T, 0.15 M	9 mL	40 mL / 5 g
Acetonitrile	3500 mL	5000 mL
Detritylation	< 1000 mL	1000 mL
Capping reagent A	19 mL	100 mL
Capping reagent B	19 mL	100 mL
DEA	40 mL	100 mL
Oxidation reagent	120 mL	1000 mL
Activator (0.3M BTT)	50 mL	250 mL

Synthesis specification for ÄKTA oligopilot plus 100

- The specified volumes are appropriate for a 6.3 mL column.
- The duration of synthesis is approximately 6 hours.
- Waste generated: 3500 mL
- Required waste container: 20 L

Note: *The acetonitrile must be extremely dry. The maximum water content in the solution should not exceed 30ppm.*

Recommended amidite concentrations

Recommended amidite concentrations for different columns using Primer Support 5G:

- 6.3 mL column, 1.2 mL column, cassette type columns: 0.1 to 0.15 mM
- Columns with volumes from 50 mL and above use 0.2 M amidite concentration.

6.4 Preparing the column reactor

About fixed column reactors

The fixed column reactors (1.2, 6.3, 12, 24, and 48 mL) are made of stainless steel with a stainless steel filter (mesh 20 µm) at each end.

Packing the column

Pack the column according to the Operating Instructions for each specific support. Below you find the packing instruction on how to pack Primer Support 5G.

Packing Primer Support 5G in fixed volume columns

Follow the steps below to pack Primer Support 5G in fixed volume columns.

Step	Action
1	Choose the support appropriate for the synthesis (the base attached to the support will become the 3'-terminal residue of the synthesized oligonucleotide).
2	Dismantle the column and fill the column tube with dry Primer Support 5G (see *Fixed volume columns, on page 77*).
3	Remove any excess support from the top edge and thread of the column tube. A small soft brush (e.g. a camera lens brush) is suitable for this purpose.
4	Place the steel filter on top of the column and the O-ring on the filter. Then screw the column adapter in place. Do not overtighten.
5	Attach the column to the ÄKTA oligopilot plus instrument. Tighten the retaining screws fingertight, then another half turn using the supplied wrench; do NOT overtighten. Make sure that the column is perpendicular and that the screws are evenly tightened.
6	Connect the tubings from the system column valve.

Step	Action
7	Start the run. Note: the support will swell within seconds and fill the column during the initial column wash.

NOTICE

Change damaged or worn PTFE seals and filters. Partly blocked filters can cause increased back pressure and high pressure alarms during a synthesis run.

Damaged PTFE seals can cause support material to leak out and block UV cell and flowrestrictor, which may also cause high pressure alarms.

NOTICE

Always check for leaks prior to starting a synthesis run.

Fixed volume columns

Weight of support and column volumes required for fixed column volumes.

Primer Support 5G (g)	Column volume (mL)
700 mg	6.3
133 mg	1.2

Packing Primer Support 5G as a slurry

This packing method is suitable when using variable adapter type columns. Prepare a slurry by adding 3 parts of acetonitrile to 1 part of Primer Support 5G. The resulting volume is 3.33 parts.

Note: *Determine the column volume using this formula:*

Column volume (mL) = 3 × 3 × 3.15 × bed height (cm)

6.5 Connecting reagents and solutions to the inlet panel

Inlet panel connectors

Connect the reagents and solutions to the inlet panel connectors according to the following table.

Reagent	Inlet panel number
Thiolation inert gas	1
Thiolation	2
Cap A inert gas	3
Cap A	4
Ox inert gas	5
Oxidation	6
Cap B inert gas	7
Cap B	8
Detrit inert gas	9
Detrit	10
ACN inert gas	11
Acetonitrile	12
Extra inert gas	13
Extra reagent	14
Activator inert gas	15
Activator	16

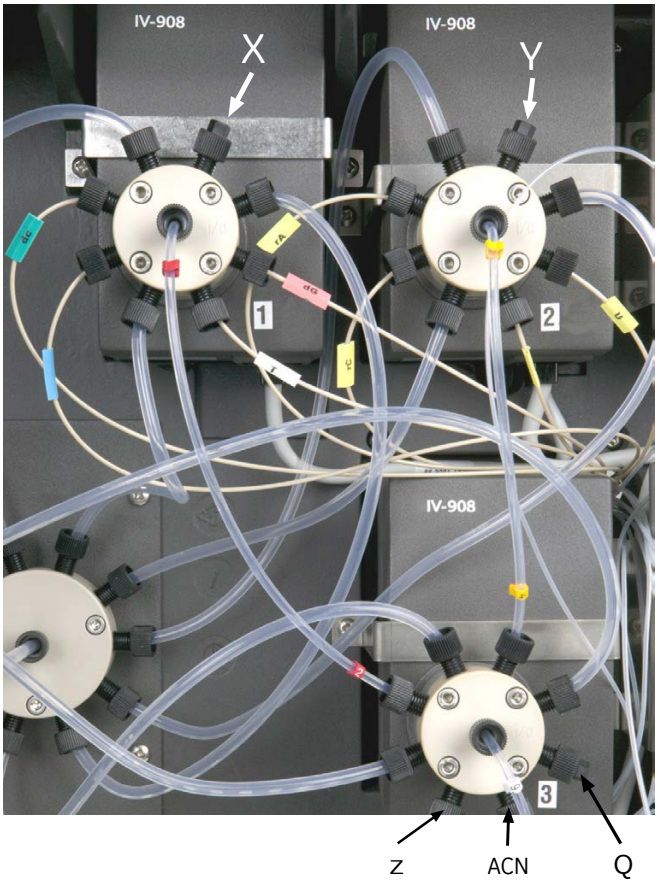
6.6 Connecting additional amidities

In this section

This section describes how to install extra slides to hold extra amidite bottles.

Standard delivery

The standard ÄKTA oligopilot plus is provided with eight amidite bottle sliders. Each slider can hold a standard 100 mL bottle. An additional four sliders can be included, giving a total of 12 amidite bottles. The picture below shows the inlets for the 4 extra amidites (X, Y, Z and Q).



Base	Connected to
X	Valve 1, position 8
Y	Valve 2, position 8

Base	Connected to
Z	Valve 3, position 4
Q	Valve 3, position 6

Note: *If amidite inlet tubing are connected to valve 3, an additional acetonitrile line must be connected between the acetonitrile inlet block and valve 3 port 5. Use tubing with an outer diameter of 3/16".*

Parts needed

Note: *The length of the tubing should be no higher than 150 cm, the shorter the better.*

- Amidite bottle slider (connectors included), product code 18113846
- PEEK tubing
  - **ÄKTA oligopilot plus 10**: orange PEEK, OD 1/16", ID 0.50 mm
  - **ÄKTA oligopilot plus 100**: beige PEEK, OD 1/16", ID 1.0 mm
- ETFE tubing, OD 1.16" (for inert gas)
- Ferrule, ID 1/16"
- Connector, 5/16"
- Bottel cap (for attaching liquid and inert gas tubing to GL-45 threaded bottles) for bottles > 100 mL: Bottel Cap product code 18113701

Connecting the amidite bottle slider

Follow the steps below to connect the amidite bottle slider.

Step	Action
1	Connect the inert gas tubing (O.D. 1/16") to the right-hand gas manifold (B3) on the inside of the door of ÄKTA oligopilot plus.
2	Unscrew one of the stop plug connectors, and place a fingertight connector with tubing in its place.
3	Connect the tubing from the fingertight connector to the new amidite bottle.
4	Connect the ETFE tubing with a fingertight connector to the vial holder. ***Note:*** *If a GL-45 amidite bottle is used, connect the tubing to the connector marked **G** using a 5/16" connector.*

Inspection of inert gas leakage

Always check for inert gas leakage prior to starting a synthesis run. To verify that there is no inert gas leakage, follow the steps below.

Step	Action
1	Switch off the gas supply to the ÄKTA oligopilot plus.
2	Wait 2-5 minutes.
3	Check the pressure meters on the front panel of the instrument.
	Note:
	The pressure should be stable. A leakage is often indicated when the pressure decreases to 0 bar within 1 minute.

7 Operation

About this chapter

This chapter gives instructions on how to operate the product in a safe way.

Safety precautions

WARNING

Do not operate the product in any other way than described in the user documentation.

In this chapter

7.1 Operation overview

Workflow

The typical workflow in ÄKTA oligopilot plus, after turning on the system and connecting it to UNICORN, can be divided into a number of steps.

Step	Action
1	Create a method *Section 7.3 Setting up a run, on page 87*
2	Prepare the system for a run *Chapter 6 Pre-synthesis procedure, on page 66* *Section 7.3 Setting up a run, on page 87*
3	Start a run using a method *Section 7.5 Performing a run, on page 94*
4	During a run - view and change parameters *Viewing the run, on page 101*
5	Procedures after a run *Chapter 8 Post-synthesis procedure, on page 103*
6	Evaluate the results See UNICORN user documentation.

Liquid flow path

See *Appendix I Connection diagrams, on page 253* for an illustration of the liquid flow path in ÄKTA oligopilot plus.

7.2 Starting the instrument and the control system

Starting the instrument

Make sure that all external tubing to the system including the reagent inlet, amidite inlets and waste outlets are correctly connected. Check that all tubing connections are properly tightened and that all valves are connected to a tube or termination.

Follow the steps below to start the instrument.

Step	Action
1	Turn on the **Power** switch on the ÄKTA oligopilot plus instrument.
2	Set the two argon regulators, located on the corner of the instrument, to 0.3 bar.

Note: *It is important to have an equal pressure on all reagent bottles so that the pumps will deliver the flow rate set in the method.*

Starting UNICORN

Follow the steps below to start the UNICORN software.

Step	Action
1	Turn on the monitor, computer and optional printer according to the manufacturer's instructions. Wait for the computer to start up.
2	Verify that the power indicator on the CU-950 is on when the computer has been turned on.
3	Log on to Windows operating system.
4	Start UNICORN by double-clicking on the UNICORN shortcut icon on the desktop.

Step	Action
5	In the ***Logon*** dialog, select a user from the ***User name*** list and enter the password. If you log on for the very first time, select user ***default*** and enter the password ***default***. Click ***OK***.

Result:

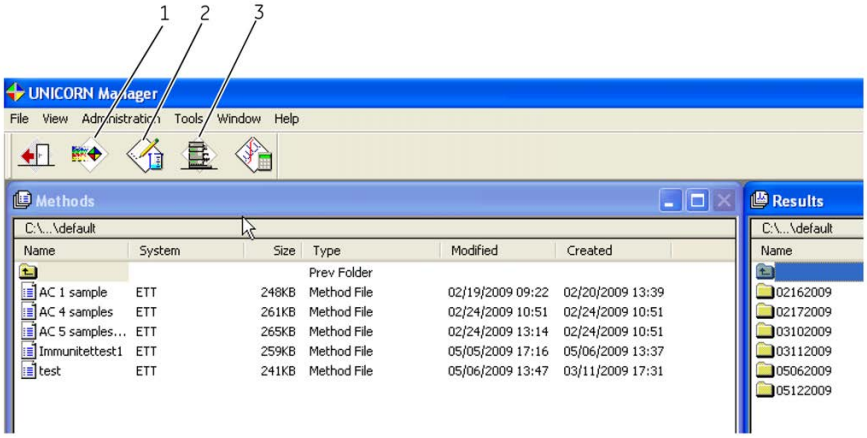
UNICORN starts and the ***UNICORN Manager*** window opens.

Note:

See the UNICORN user documentation for instructions about how to create new users.

The *UNICORN Manager* window

The illustration below shows the ***UNICORN Manager*** window.



Part	Function
1	The ***Instant Run*** icon immediately starts the system control wizard used to start a run.
2	The ***New Method*** icon opens the ***Method Editor*** module and displays the ***New Method*** dialog box.
3	The ***System Control*** icon activates the ***System Control*** module and displays the ***Manual instruction*** dialog box.

Control system in UNICORN

To open the ***System Control*** module in UNICORN, click the ***System Control*** icon in the ***UNICORN Manager*** window.

7.3 Setting up a run

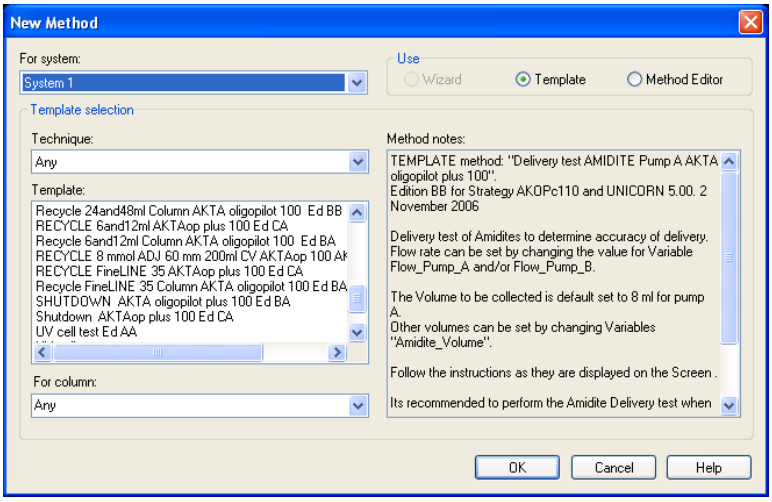
Introduction to UNICORN methods

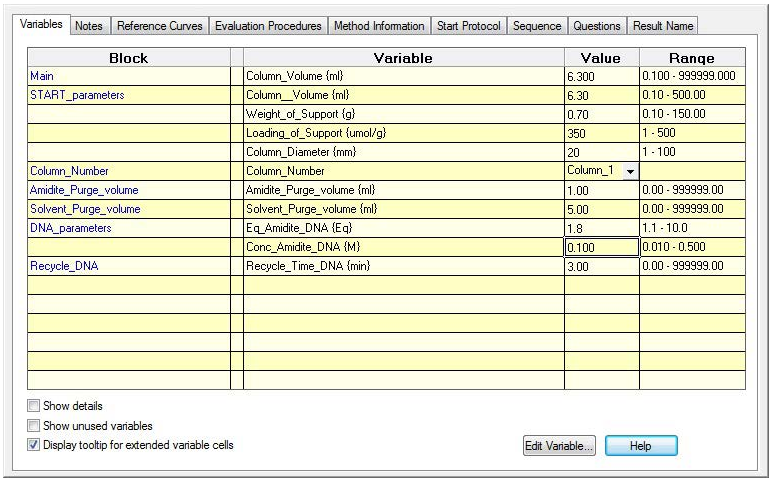
UNICORN is supplied with a number of predefined methods (called template methods). Depending on which column is used, synthesis methods come in one or two versions - one for Recirculation amidite coupling technique and one for Flowthrough amidite coupling technique. Recirculation is the recommended technique. The flowthrough technique is included because it is used in OligoProcess™ systems and will provide easy method development from ÄKTA oligopilot plus to OligoProcess in those cases.

Creating a method

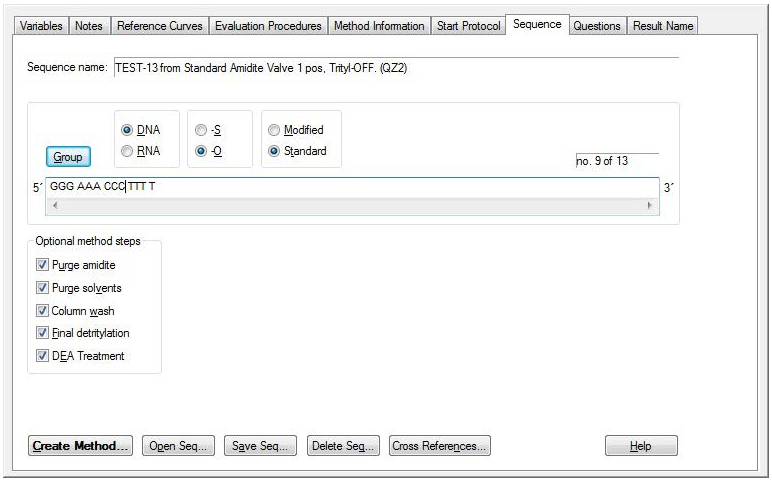
Follow the steps below to create a new method.

Step	Action
1	Click the ***New Method*** icon in the ***UNICORN Manager*** window, see *The UNICORN Manager window, on page 86*. *Result:* The ***New Method*** dialog opens.



Step	Action
2	• For the ÄKTA oligopilot plus 10 system, select the ***Recycle 1 ml Column 10*** template method. • For the ÄKTA oligopilot plus 100 system select the ***Recycle 6 ml Column AKTA oligopilot 100*** template method. All methods have information regarding the method displayed in the ***Method notes*** window. ***Note:*** *The column list is not used in oligonucleotide applications.* ***Note:*** *Techniques not used should be set to **Any**.*
3	Click ***OK*** to open the selected template method.
4	In ***Method Editor***, choose ***View*** →***Run setup***, or click on the ***Run Setup*** icon.  *Result:* The ***Run Setup*** dialog tabs are opened to the default tab (***Variables***). 

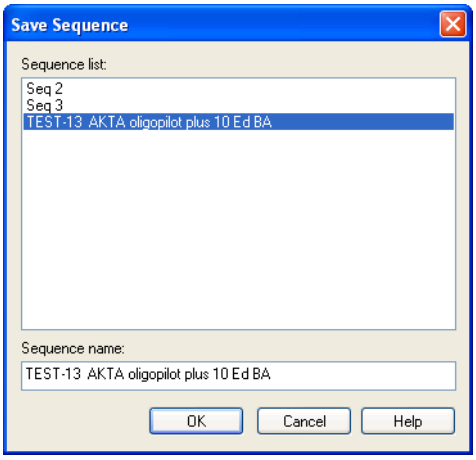
Step	Action
5	Click on the ***Sequence*** tab.



Result:

The ***Sequence name*** and sequence are displayed.

Step	Action
6	To change the name of a sequence, click ***Save Seq***. The ***Save Sequence*** dialog opens where a new name can be entered.



The same procedure is used if a new sequence is entered. Enter the sequence from 5' to 3' and click ***Save Seq***.

Note:

This saves the sequence in a sequence list, not as a synthesis method.

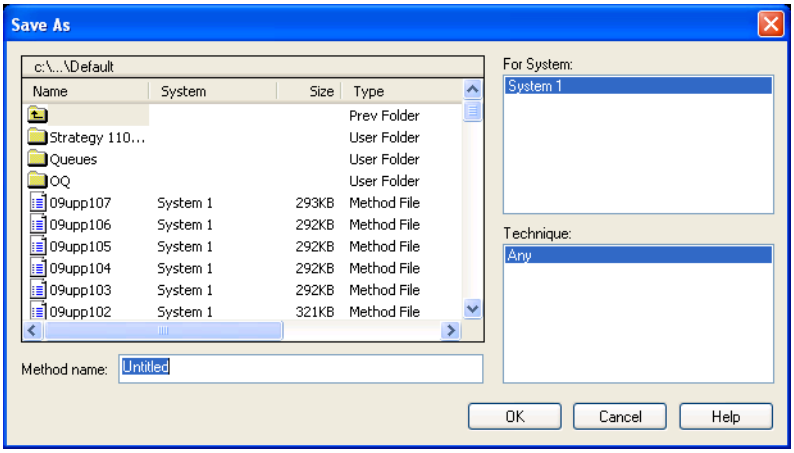
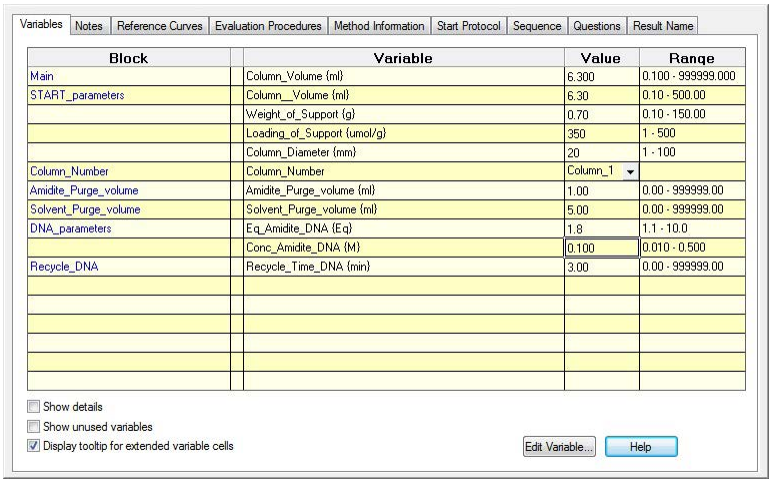
The sequence can be modified using the radio buttons to signify:

In the ***Optional method steps*** area:

- ***Final detritylation***, should have a check mark if the synthesis is Trityl-Off and no checkmark if it is a Trityl-On synthesis.
- Active checkmarks are always recommended for:
  - ***Purge amidite*** (amidites + tetrazole/activator).
  - ***Purge solvents*** (capping + oxidation and/or thiolation reagent).
  - ***Column wash***.

Note:

*When making your first run, all **Optional method steps** should have a check mark activated.*

Step	Action
7	When the sequence and ***Optional method steps*** have been set, click ***Create Method***. *Result:* The ***Save As*** dialog opens. 
8	Enter a synthesis method name, for example, *Test13*. Technique ***Any*** is the default selection. Do not select any of the other techniques.
9	Click ***OK*** to save the synthesis method. Click on the ***Variables*** tab. 
10	Enter the ***Weight_of_Support*** calculated when packing the column.

Step	Action
11	Enter the appropriate ***Loading_of_Support***.
12	Verify that ***Conc_Amidite_DNA*** is set to the concentration of the amidites in the amidite bottles.
13	Choose ***File*** →***Save*** or click on the ***Save*** icon to save the method, which is now ready to use for a synthesis run. 

7.4 Preparations before start

Before the first run

Before starting the first run, make the following preparations:

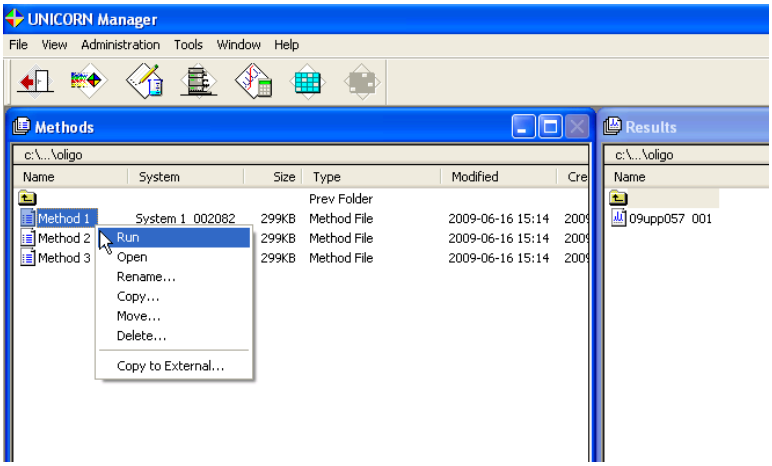
- Connect all external tubing to the system including the reagent inlet, amidite inlets and waste outlets.
- Pack a column with Primer Support 5G.
- Attach the column to a column holder on the instrument.
- Connect the reagents and amidites.
- Purge all reagent and amidite lines.

7.5 Performing a run

Select a method

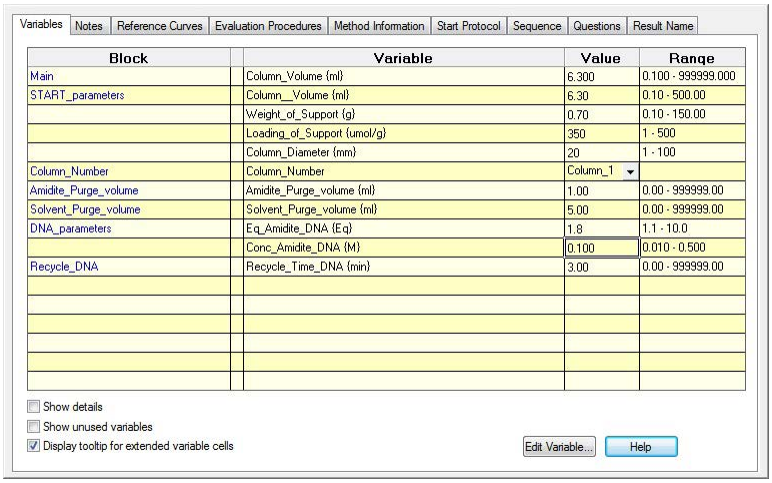
Follow the steps below to select a method.

Step	Action
1	In the ***UNICORN Manager*** window, click on the method you wish to use with the right mouse button, then click on ***Run***.

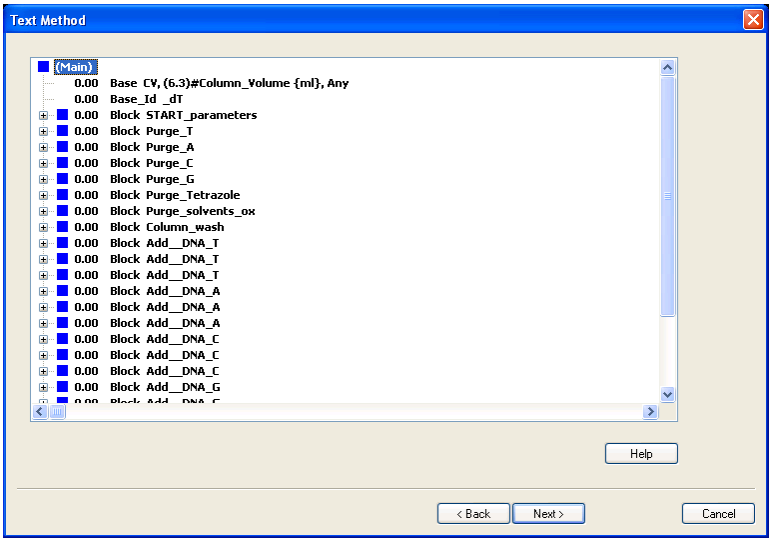


After a few seconds the first page of the Start Protocol will appear. During the synthesis start procedure you will go through the Start Protocol, pages selected in the synthesis method to be shown during start of a synthesis run. You will recognize the pages from the ***Method Editor***.

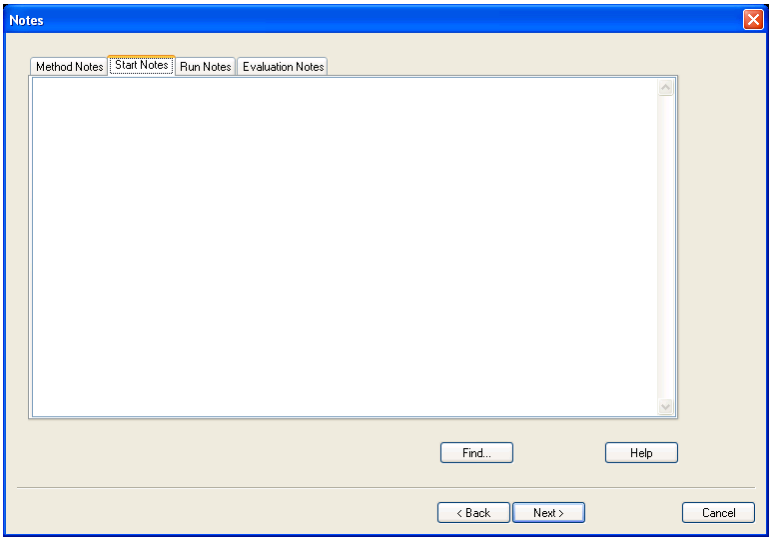
Step	Action
2	In the ***Variables*** page, verify the values entered for variables ***Weight_of_support*** and ***Loading_of_support***. ***Note:*** *For ÄKTA oligopilot plus 10 systems only the scale is entered.*



3	Click ***Next*** to proceed to the next page.
4	The ***Text Method*** page opens but can not be modified at this stage. However, proofreading the sequence is recommended since what is executed during the synthesis is what is programmed in the text method. The ***Sequence*** page is used to create the text method.

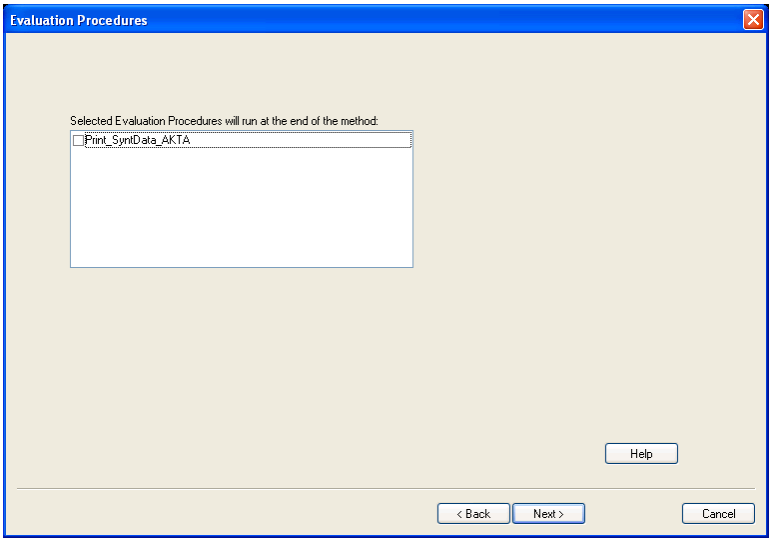


Step	Action
5	Click ***Next***.
6	In the ***Start Notes*** field in the ***Notes*** page, the user can add information regarding the synthesis. The notes are stored as part of the synthesis result file and can be reviewed after completion of the synthesis.

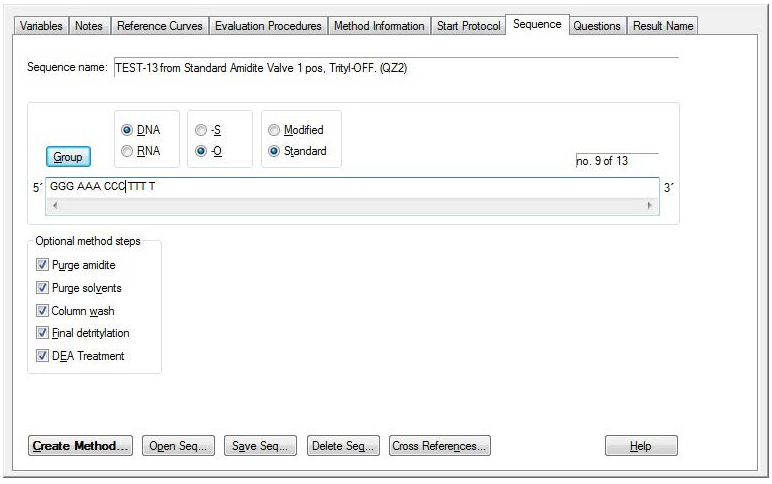


7	Click ***Next***.

Step	Action
8	In the ***Evaluation Procedures*** page, select an evaluation procedure and a synthesis report will be printed automatically at the end of the synthesis. A synthesis report can also be printed manually during evaluation.

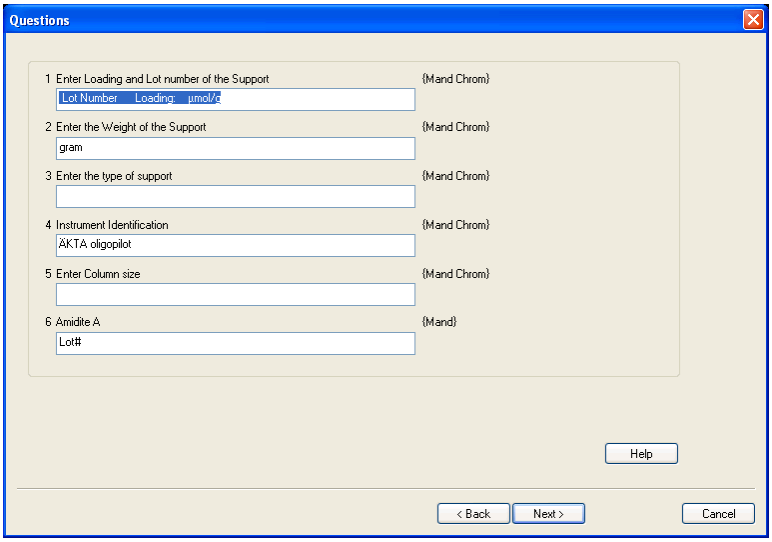


9	Click ***Next***.
10	In the ***Sequence*** page, the sequence and ***Optional method steps*** are displayed but can not be changed.

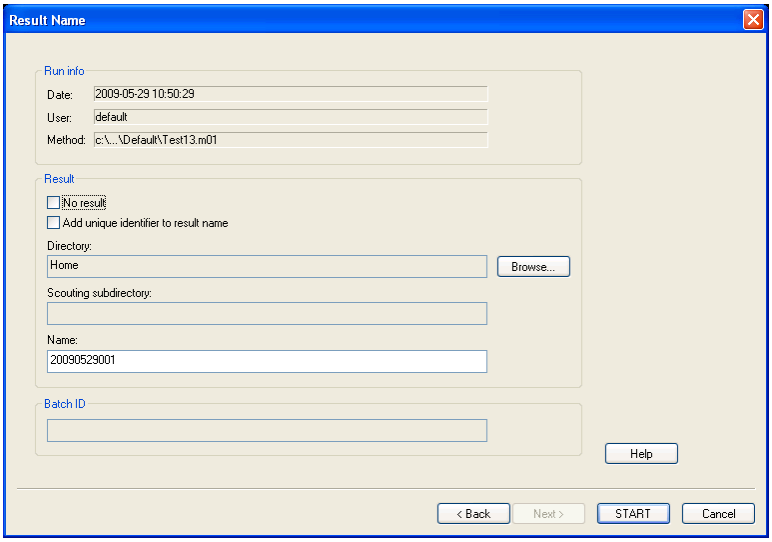


11	Click ***Next***.

Step	Action
12	The ***Questions*** page opens. The answers and questions from this page will be included in the Synthesis Report. The questions do not affect the execution of the synthesis method. Mandatory questions must be answered before continuing.

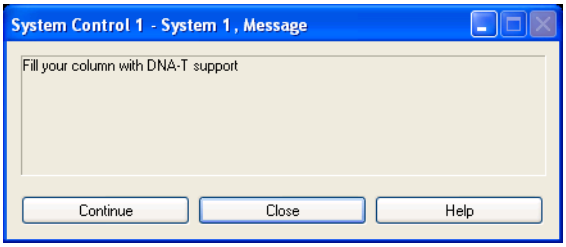
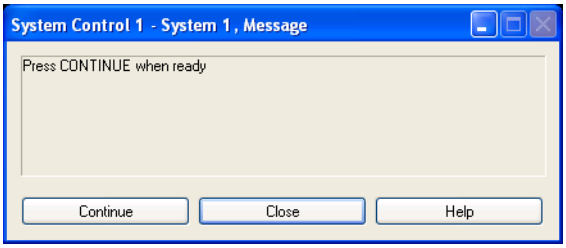


13	Click ***Next***.

Step	Action
14	The ***Result Name*** page opens. The default synthesis result file name is the date (in digits) + 01, where 01 is added in case you perform several syntheses during the same day (in which case, the suffix on the next run will be 02, and so on).  You can add text to the default name or enter a different result name. If you wish to store the result file in a specific folder, click ***Browse*** to locate the folder and double click on it. Then click ***OK***.
15	Verify the path under ***Directory***.

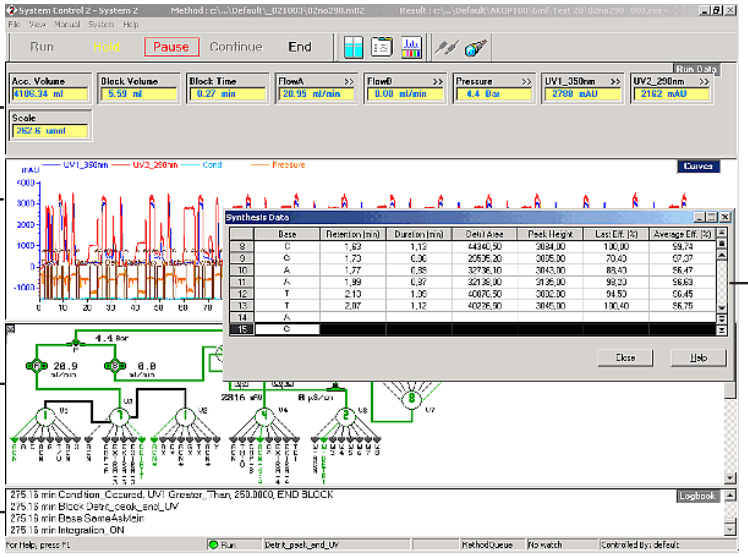
Start the run

Follow the steps below to start the run.

Step	Action
1	After entering a name and directory path, click ***Start***. *Result:* The synthesis run starts. The first thing that occurs is that the run is set to ***Pause*** and the following message is displayed. 
2	Read the message and confirm by clicking ***Close***. Another message is displayed. 
3	Read the message and confirm by clicking ***Continue***. *Result:* The system is now running.

Viewing the run

The progress of the run can be viewed in detail in the ***System Control*** module. During the synthesis the following view panes can be displayed: ***Run Data***, ***Curves***, ***Flow scheme*** and ***Logbook***.



No.	Name	No.	Name
1	***Run Data***	4	***Logbook***
2	***Curves***	5	***Synthesis Data***
3	***Flow scheme***		

The online integrated trityl values showing the coupling efficiency can be displayed by selecting ***View*** →***Synthesis Data***. ***Duration*** and ***Retention*** are displayed as mL or min, depending on what has been chosen for the X-axis in the ***Curves*** view pane. To change between mL or min, click on the X-axis.

The highlighted row in ***Synthesis Data*** shows which cycle is currently running.

Customize the view panes

To customize the view panes, right-click in the respective view pane and select ***Properties***. For more information about customizing the view panes, see the UNICORN user documentation.

Ending the run

To stop the run on a system before it is finished:

Click ***End*** above the ***Run data*** view pane.

Status indicator colors

The status indicator is located at the bottom of ***System Control***.

The table below shows how the indicator colors relate to the run status.

Indicator color	Run status
White	End
Green	Run or Manual
Yellow	Hold
Red	Pause

Error indication

When a warning or an alarm is issued from a system, an error code is displayed.

Evaluate the results

See UNICORN user documentation for how to evaluate the results.

8 Post-synthesis procedure

About this chapter

This chapter describes the steps that should be followed to recover the DNA oligonucleotide product from the support and to remove the protecting groups from the product.

Note: *This chapter is valid for Primer Support 5G and might not be applicable for other supports. Always consult the instructions provided with the solid supports used.*

Safety precautions

WARNING

Hazardous substances. When using hazardous chemicals, take all suitable protective measures, such as wearing protective clothing, glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the product.

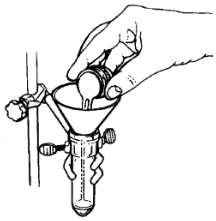
Procedure

After a completed synthesis, follow the steps below.

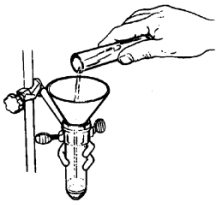
Step	Action
1	After a completed synthesis, detach the column (reactor) from the column holder.
2	Place the reactor on a vacuum bottle with adapter (without removing the reactor top). Apply vacuum for >30 minutes to dry the support. The increase in column weight should be at least 1 gram for the 6.3 mL column and 0.2 gram for the 1.2 mL column. This weight increase assumes that Primer Support 5G is used to make the Test13 oligonucleotide.

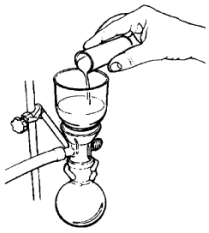
Step	Action
3	Unscrew the reactor top while vacuum is still applied. Release the vacuum and remove the reactor from the vacuum filter.

4	Using a spatula, transfer the support to a bottle or tube. To resist exposure to the deprotection conditions, the bottle or tube should have a PTFE seal. After use, clean the column reactor by sonication for 30 minutes in methanol. Dry the reactor thoroughly before re-using.



5	Using a spatula, transfer the support to a bottle or tube. To resist exposure to the deprotection conditions, the bottle or tube should have a PTFE seal. ***Note:*** *After the use, clean the column reactor by sonication for 30 minutes in methanol. Dry the reactor thoroughly before re-using.*
6	Add minimum 10 mL ammonium hydroxide per gram support. Shake vigorously and incubate overnight at 55°C.



Step	Action
7	Allow the reaction mixture to cool before opening the bottle or tube, then filter the contents through a glass-filter (pore size 3-4). Use a rotary evaporator flask to collect the filtrate. 
8	Wash the support with minimum 5 times the column volume 1:1 ethanol:water.
9	Add 0.5-1 volumes of ethanol to reduce bumping, then evaporate the filtrate to dryness on a rotary evaporator.
10	Re-dissolve the oligonucleotide product as required.

9 Maintenance

About this chapter

This chapter provides information to enable users and service personnel to clean, maintain, calibrate and store the product.

Important

Regular maintenance is important for safe and trouble-free operation of your instrument. The user should perform daily and monthly maintenance. Preventive maintenance should be performed on a yearly basis by qualified service personnel. For maintenance of a specific component, carefully read the component manual and follow the instructions.

Safety precautions

WARNING

Before attempting to perform any of the procedures described in this chapter, you must read and understand all contents of the corresponding sections in the Safety instructions chapter as listed below:

- *General precautions, on page 14*
- *Personal protection, on page 15*
- *Maintenance, on page 21*

In this chapter

9.1 User maintenance schedule

Introduction

The user maintenance schedule provides a guide to maintenance operations and intervals at which these operations should be performed by the user. The user is however responsible for deciding the type of operations and length of intervals necessary to maintain system function and safety.

User maintenance schedule

Interval	Action	Instructions/reference
Daily	Leak inspection	• Visually inspect the system for leakages. • Inspect the P-900 pumps for leakage. ***Note:*** *If the rinsing liquid is discolored after less than 20 runs, replace the piston seals.*
	Wash the system flow path	1. For cleaning the flow path, see *Cleaning the system flow path, on page 111*. 2. For leaving the system for a few days, see *Section 9.8 Storage, on page 136*.
	Calibrate pH electrode (optional)	Calibrate the pH electrode (if applicable) according to *Monitor pH/C-900 Operating Instructions*, product code 29054925.
Weekly	Check inlet filters	Check the inlet filters visually and replace them if necessary. Check the connectors in the bottle cap and on the inlet panel for tightness.
	Bottle and vial filters (frits)	Replace the frits. ***Note:*** *Do not use frits in bottles with chlorinated solvents. The filter part will swell and come loose.*
	Replace on-line filter (if applicable)	Replace the on-line filter.

Interval	Action	Instructions/reference
	Change pump rinsing solution	Change rinsing solution. Always use acetonitrile as rinsing solution. If the volume of rinsing solution in the storage bottle has increased, it can be an indication of internal pump leakage. Replace the piston seals according to *Section 9.4.2 P-900 Replacing the piston seal, on page 120*. If the volume of rinsing solution in the storage bottle has decreased significantly, check if the rinsing system connectors are mounted properly. If the rinsing system connectors are not leaking, the rinsing membranes or piston seals may be leaking. Replace the membranes and piston seals according to *Section 9.4.2 P-900 Replacing the piston seal, on page 120*. ***Note:*** *Change any older amidite solutions. This maintains the quality of the solutions used.*
Monthly	Flow restrictor	Check that flow restrictor generates the following back-pressure: FR-902: 0.2 ±0.05 MPa Check the back-pressure as follows: 1. Disconnect the flow restrictor. 2. Connect a tubing (approx. 1 m, i.d. 1 mm) to a free port in the injection valve. Set the valve manually to this port. Put the open end in a waste container. 3. Run the pump at 10 mL/min with acetonitrile. Note the back-pressure (Bp1) on the pump display, or in the ***Run Data*** window. 4. Connect the flow restrictor to the open end of the tubing (observe the IN marking). Put the flow restrictor in the waste container. 5. Run the pump at 10 mL/min with acetonitrile. Note the back-pressure (Bp2) on the pump display, or in the ***Run Data*** window. 6. Calculate the back-pressure generated by the flow restrictor. Replace it if it is not within limit.

Interval	Action	Instructions/reference
	Inert gas manifold	Inspect the pressure relief valve for leakage. ***Note:*** *As a first measure, pull out the relief valve and release it. If necessary, clean the O-ring using ethanol and a soft cloth.*
	Check Monitor UV-900	Check the Monitor UV-900 instrument according to the instructions in the *Monitor UV-900 Operating Instructions*, product code 28962214.
Yearly	Valves inspection	Check for external or internal leakage. Replace channel plate and distribution plate yearly or when required. Refer to the relevant valve instruction sheet.
	Bottle caps for Capping A and DEA bottles	Replace the sealings.
When required	Replace membranes or rocker	Refer to the relevant User manual.

9.2 Cleaning

Cleaning before planned maintenance/service

To ensure the protection and safety of service personnel, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts maintenance work.

Please complete the checklist in the *On Site Service Health and Safety Declaration Form* or the *Health and Safety Declaration Form for Product Return or Servicing*, depending on whether the instrument is going to be serviced on site or returned for service, respectively.

Health and safety declaration forms

Health and safety declaration forms are available for copying or printing in the *Reference information* chapter of this manual, or on digital media supplied with the user documentation.

Cleaning the system flow path

Rinse the system with acetonitrile using the shutdown template method provided.

Cleaning the optical fibre connectors

Refer to the relevant section in the *Monitor UV-900 Operating Instructions*.

Cleaning the Monitor UV-900 flow cell

If the autozero level is higher than 1000 mAU at 280-290 nm, the cell must be cleaned

- to pass an installation test (step gradient)
- to get appropriate detrit values during synthesis

The procedure takes 30 minutes from start to end.

WARNING

Hazardous substances. When using hazardous chemicals, take all suitable protective measures, such as wearing protective clothing, glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the product.

Required items

The table below lists the required items.

Item	Amount
MilliQ or de-ionised water	100 mL
Nitric acid, 69-70%	20 mL
ETFE tubing, O.D. 1/16", I.D. 1 mm (18114238)	20 cm
Fingertight connector, 1/16" (18111255)	1
Male/luer female connector, 1/16" (18111251)	1
10 mL syringe	2
Beakers	3

Procedure

Follow the steps below to clean the UV-900 flow cell.

Step	Action
1	Connect the male luer to the UV cell inlet.
2	Connect the Fingerthight 1/16" connector via the ETFE tubing to the UV cell outlet.
3	Connect an empty 10 mL Syringe to the luer to draw water, air and cleaning solutions.
4	Place the ETFE tubing into a beaker with water.
5	Pull 8-10 mL of water into the syringe.
6	Pull 8-10 mL of air into the syringe, to remove the water.
7	Pour the nitric acid in a beaker and place the ETFE tubing in it.
8	Pull 8-10 mL of cleaning solution into the syringe.
9	Leave the syringe and cleaning solution in place for 30 minutes.
10	Pull 2-3 mL of air into the syringe.
11	Remove the syringe containing the cleaning solution.
12	Connect the empty 10 mL syringe to the luer.
13	Place the ETFE tubing in a beaker with water.

Step	Action
14	Pull 10 mL of water into the syringe. Do this twice.
15	Pull 8-10 mL of air into the syringe to remove the water.
16	Reconnect UV cell to system.
17	Rinse the UV cell by pumping acetonitrile to waste.

9.3 Component maintenance

In this section

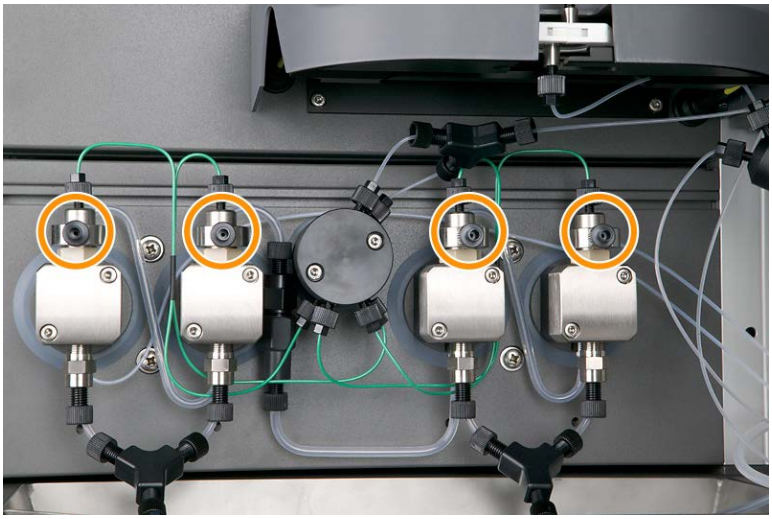
9.3.1 Introduction

In this section the maintenance of P-900 pump is described. Maintenance and preventive replacement of parts of the other major components are described in the respective manuals included in the system documentation.

The system documentation also includes a spare part list to be used to find common spare parts and their code numbers for ordering. This list can also be found online at cytiva.com/oligo.

9.3.2 Purging the P-900 pump

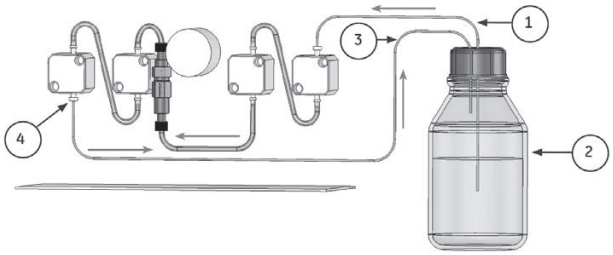
Follow the steps below to purge the pump.

Step	Action
1	P-900 pump has four purge valves. When purging, open one of the valves with ½ to 1 turn counterclockwise. *Result:* *Result:* Liquid will stream out through the vent of the valve, assuming that the reagent bottles are pressurized. 
2	Wait until a steady flow of solvent is coming from the purge valve. This can take a couple of minutes.
3	If the inlet lines are too long it may be necessary to connect a 20-60 mL solvent resistant syringe to the P-900 purge valve and help the solvent in to the pump using the syringe.
4	When a steady flow is established, close the purge valve and continue with the remaining 3 purge valves.

9.3.3 Priming the piston seal rinsing system of the P-900 pump

Follow the steps below to prime the piston seal rinsing system of the P-900 pump.

Step	Action
1	Connect the tubing and the check valve according to the image below. Note the flow direction on the check valve.



2	Immerse the rinsing tubing (1) through the membrane in the included bottle cap in a flask (2) containing 100% acetonitrile.
3	Connect a syringe with the included connectors to the rinsing tubing (3) that is connected to the underside of the left pump head on pump A (4). Slowly draw 5-10 mL rinsing solution into the syringe. ***Note:*** *The material inside the luer to 1/16" female adapter is not compatible with acetonitrile. Make sure that immersion occurs only for very short periods of time.*
4	Remove the syringe and insert the tubing (3) through the bottle cap membrane into the bottle. Make sure the tubing is approx. 1 cm above surface of the liquid as this will make it easier to observe that the rinsing system functions as expected during synthesis.
5	When the liquid/acetonitrile level in the bottle is low then add solvent until it is 1 cm below tubing 3. The solvent should be changed when it's discolored (brownish, yellowish colour). If the solvent get discoloured in less then a week then it might be time for a service check and replacement of Pump seals.

9.4 Disassembly and assembly of components and consumables

Important

The operator must carefully read and understand the instructions supplied for each component before disassembly and assembly of the component. When replacing consumables, such as tubing and tubing connectors, all necessary safety precautions must be taken. Contact your local Cytiva representative if further information or help is needed.

In this section

9.4.1 Safety precautions

WARNING

Disconnect power. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.

WARNING

Before disassembly, check that there is no pressure in the piping system.

WARNING

Before operation, all process connections and the piping system must be tested for leakage at maximum pressure for continued protection against injury risks due to fluid jets, burst pipes or potentially explosive atmosphere.

9.4.2 P-900 Replacing the piston seal

Introduction

If there are signs of liquid leaking between the pump head and the housing side panel or the volume of the rinsing solution has increased or decreased, replace the piston seal of the leaking pump head.

NOTICE
Do not disassemble the pump head unless there is good reason to believe that the seal is leaking. Always ensure that sufficient spare components are available before attempting to replace the piston seal. It is not possible to reinstall a used piston seal after removal.

Note: *The power must be switched OFF when removing and refitting the pump heads.*

Note: *Always replace the piston seals on both pump heads at the same time. An even better practice is to replace all four piston seals.*

Spare parts and tools required

Seal kit containing (see cytiva.com/oligo for code no.):

- 2 or 4 piston seals
- 2 or 4 rinse membranes
- 1/4 inch wrench (supplied with the pump)
- 3 mm hex key (supplied with the pump)
- Screwdriver (supplied with the pump)

Note: *After a new seal has been installed, the pump should be run in, see Running-in a new piston seal, on page 123.*

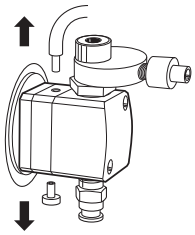
Note: *Before disassembling the pump heads move all input buffers bottles below the level of the pump heads to prevent siphoning.*

NOTICE
Read the following instructions carefully. The individual parts of the pump head can be assembled incorrectly. Take care to ensure that the orientation of each part is correct before continuing with the next instruction.

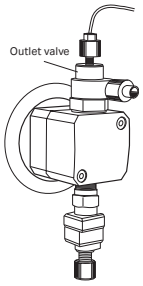
Instructions for removing the piston seal

Follow the steps below to remove the piston seal of a pump:

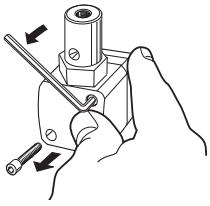
Step	Action
1	Switch off the pump at the mains power switch on the back panel. Remove the piston seal rinsing system. The connectors are simple plug-in fittings.



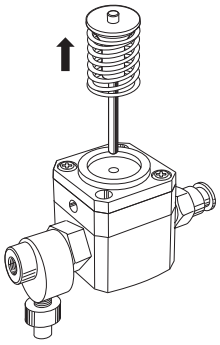
2	Completely loosen the tubing connector on the outlet valve.



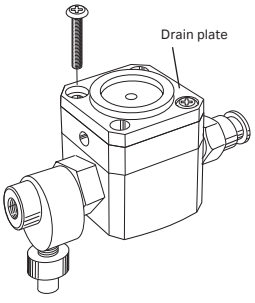
3	Using the hex key, unscrew and completely remove one of the two hex screws locking the pump head in position.

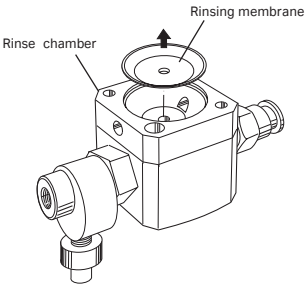


Step	Action
4	When unscrewing the second locking screw, push firmly on the front face of the pump head to compensate for the pressure of the piston return spring. Hold the pump head firmly to prevent it from twisting. Remove the second screw and, without allowing the pump head to twist sideways, carefully pull it out.

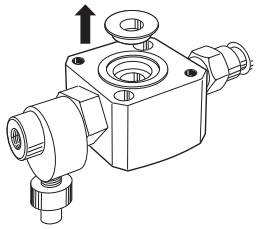


5	Place the pump head face down on the bench. Pull out the piston together with the return spring.
6	Inspect the piston and return spring for sign of damage. If damaged, they should be replaced.
7	Wipe the piston with a clean cloth. If salt solutions have been used the piston may be slightly corroded. This corrosion can be removed with a rubber eraser. If it cannot be wiped or rubbed clean, scrape off any deposits with a scalpel or razor blade. Inspect the piston with a magnifying glass for scratches. Replace with a new piston if any scratches or cracks are found.
8	Remove the two screws securing the drain plate and the rinse chamber. Remove and discard the rinsing membrane. Remove the rinse chamber. For P-903, remove also the support washer.





Step	Action
9	Gently withdraw the piston seal. Discard the used seal.



10	The pump head, rinse chamber and drain plate should be carefully rinsed or cleaned in an ultrasonic bath, if available. If dirt can be seen on any surfaces, the inlet and outlet check valves should be removed and cleaned separately (see *Section 9.4.4 Removing and cleaning the inlet and outlet check valves, on page 130*).

Proceed to *Installing the piston seal, on page 127*. Refer to *Exploded views of the P-901 and P-903 pumps, on page 126* for details of the pump.

Running-in a new piston seal

Note: *This section is not applicable for Oligonucleotide synthesis.*
The piston seal should be run-in using 100% methanol.

NOTICE
To protect the pump seals, always ensure that there is a constant supply of eluent. The pump should never be allowed to run dry.

Step	Action
1	Make sure that the reservoir is filled with sufficient eluent. Immerse the inlet tubing in the eluent. The reservoir should be placed at least 30 cm above the pump inlet.
2	Connect a male Luer syringe of about 30 mL to the open end of the purge tubing.
3	Connect the male Luer connector at the other end of the purge tubing to the left purge valve at pump module module A.
4	Turn the purge valve counterclockwise half a turn to open it and slowly draw eluent to the syringe.

Step	Action
5	When fluid starts to enter the syringe continue to draw a few millilitres before closing the purge valve. Check that there is no air left in the inlet tubing.
6	Repeat steps 3 to 5 for pump module B, if fitted.
7	Check that the outlet tubings are not blocked.
8	Connect a thin capillary or a column that will give sufficient back pressure.
9	Run the pump at the following flow rate for 15 minutes: **P-901** 1 mL/min (or 2 mL/min 50%B) **P-903** 0.1 mL/min (or 0.2 mL/min 50%B)
10	Run the pump at the following flow rate, backpressure and duration: **P-901** 20 mL/min (or 40 mL/min 50%B) at a backpressure of 2 to 5 MPa for 15 minutes. **P-903** 2 mL/min (or 4 mL/min 50%B) at a backpressure of 5 to 10 MPa for 2 hours, or longer if possible (e.g. overnight).
11	Finally, change the eluent according to the instructions in *Changing eluent, on page 124*.

Changing eluent

NOTICE
To prevent precipitation of crystals when changing from a salt-containing buffer to organic solvent, always flush through the system with water as the intermediate liquid.

When changing from one eluent to another, it is extremely important that the two eluents are totally miscible with one another. If the two eluents are immiscible, the pump should be flushed first with an intermediate liquid, which is miscible with both eluents. Failure to do this will cause a wrong flow of eluent from the pump.

When changing from a salt-containing buffer to an organic solvent, use water as the intermediate liquid to prevent precipitation.

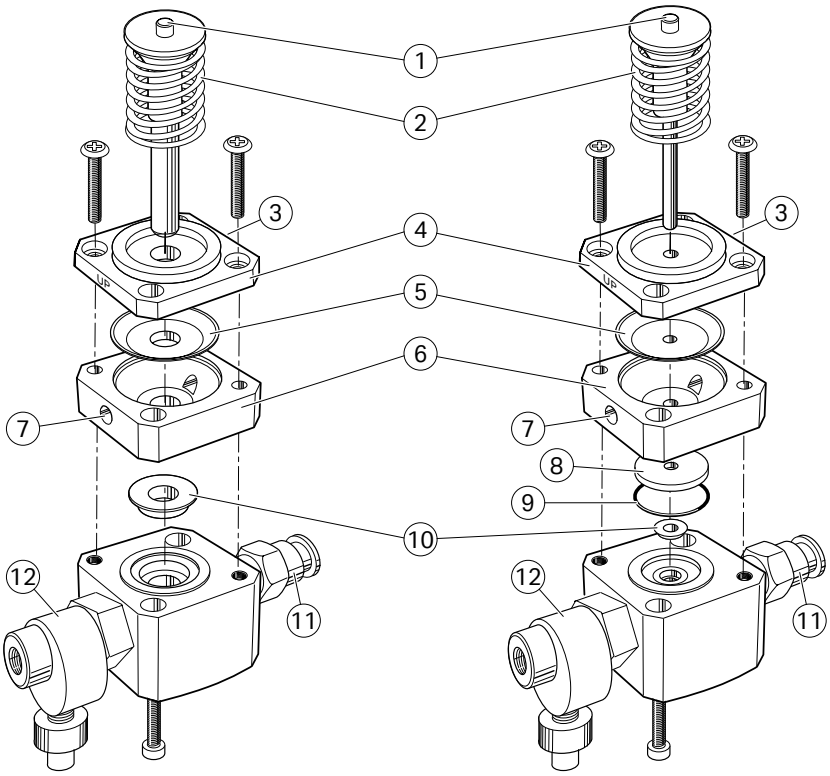
Step	Action
1	Stop the pump by setting it in ***Pause*** mode.

Step	Action
2	Transfer the inlet tubing into the new eluent or into the intermediate liquid.
3	Run the pump at a flow rate and time as specified in the table below.

Pump	Flow rate	Time
P-901	40 mL/min	10 minutes
P-903	4 mL/min	10 minutes

4	Stop the pump. If an intermediate liquid is being used, transfer the inlet tubing into the final eluent and repeat step 3 with the new eluent.
5	In UNICORN, select instruction ***PumpWash*** in ***System Control*** →***Manual*** →***Pump***

Exploded views of the P-901 and P-903 pumps



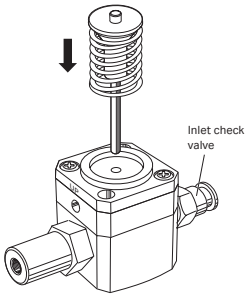
P-901 P-903

Part	Description
1	Piston
2	Return spring
3	Drainage hole
4	Drain plate
5	Rinse membrane
6	Rinse chamber
7	Rinse chamber outlet
8	Support washer
9	Jointing ring
10	Piston seal
11	Inlet check valve
12	Outlet check valve

Installing the piston seal

Follow the steps below to install a new piston seal in a pump:

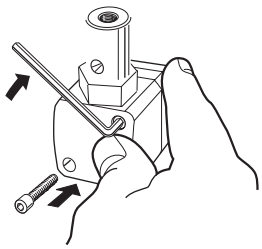
Step	Action
1	Wet the new seal slightly and place it in the hole on the pump head and press it down into position with a hard flat object For P-903, refit the support washer on top of the new seal.
2	With the pump head still facing downwards on the bench, place the rinse chamber onto the head with the rinse ports in line with the inlet and outlet check-valves. The conical depression in the rinse chamber should be facing upwards, ready to accept the new rinsing membrane. Fit the rinsing membrane with the conical face downwards.
3	Place the drain plate on top of the assembly. Use the two screws to lock the complete assembly together. ***Note:*** *Align the drainage hole in the drainage plate with the inlet check valve (the opposite side of the pump head marked* **OUT/UP***).*
4	Wipe clean the piston and remove all finger prints. Wet the piston and then insert it into the return spring. With the pump head facing downwards on the bench, insert the piston into the pump head by pushing it gently but firmly vertically downwards into the seal.



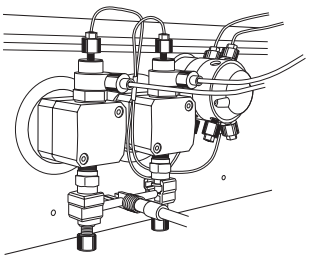
NOTICE
Do not push the piston at an angle to the head and DO NOT twist the piston.

Step	Action
5	Turn the head so that the inlet valve and drainage hole are facing downwards and the text **UP**/**OUT** on the pump head is facing upwards. Mount the complete pump head over the locating pins on the front panel. Locate the metal end of the piston and the spring towards the drive cam. Hold the pump head firmly against the side panel of the housing with one hand. Do not allow the assembly to twist under pressure from the return spring. Using the hex key, fit and tighten one of the hex screws. Fit and tighten the remaining hex screw.

WARNING
Incorrectly fitted tubing may loosen, causing a jet of liquid to spray out. This is especially dangerous if hazardous chemicals are in use. Connect the tubing by first inserting the tubing fully, then tightening the connector fingertight. Finally tighten the connector a further 1/4 turn using the key supplied.



6	Reconnect the outlet tubing to the outlet check valve and the manifold block as described above.



7	Refit the tubing of the piston seal rinse system.
8	The pump should now be purged and the new piston seal carefully run in, following the instructions in *Running-in a new piston seal, on page 123*.

9.4.3 Replacing a damaged piston

Typical symptoms of a damaged piston are observed as excessive piston seal wear, unstable pressure, a reduction in the flow or, in some cases, noise as the piston moves. The piston should be removed, examined for damage or salt precipitation and then replaced with a new piston if necessary.

If a damaged piston has been in operation, the piston seal will be destroyed and should also be replaced. To replace the piston and the seal follow the instructions in *Section 9.4.2 P-900 Replacing the piston seal, on page 120*.

In addition to the spare parts listed in *Section 9.4.2 P-900 Replacing the piston seal, on page 120*, the following are required:

Product	Product name
11-0003-34	Pump seal inert (10 mL)
11-0008-85	Pump seal inert (100 mL)

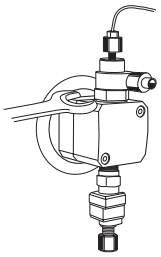
9.4.4 Removing and cleaning the inlet and outlet check valves

Before removing the check valves, move all input buffers bottles below the level of the pump heads, to prevent siphoning.

WARNING

Hazardous substances. When using hazardous chemicals, take all suitable protective measures, such as wearing protective clothing, glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the product.

Note: *Change solvent to methanol and flush out all salt before removing the check valves.*



Tools required: A 13 mm and a 1/4 inch wrench.

Step	Action
1	If the condition of the check valve is not improved by in-place cleaning, disconnect and remove the inlet manifold and outlet tubing.
2	Use the 13 mm wrench to remove the valve from the pump head.

NOTICE

Handle the check valves with care when they have been removed from the pump heads to prevent loss of any internal components.

3	Immerse the complete valve in methanol and place in an ultrasonic bath for some minutes. Then repeat the ultrasonic bath with distilled water.

Step	Action
4	Refit the check valves. The inlet check valve (with a lip for the manifold and a larger diameter opening) is fitted to the side marked **IN** of the pump head. Tighten the valves until fully finger-tight and then use the 13 mm wrench to tighten a further 1/3 of a turn (120°). Do not overtighten the valves since damage to the internal components can occur.
5	Connect the tubing by first inserting the tubing fully, then tightening the connector fingertight. Finally tighten the connector a further 1/4 turn using the key supplied.

WARNING

Before operation, all process connections and the piping system must be tested for leakage at maximum pressure for continued protection against injury risks due to fluid jets, burst pipes or potentially explosive atmosphere.

6	Refit the outlet tubing and the inlet manifold.
7	Purge the pump carefully and check that the pumping action has been corrected. See *Section 9.3.2 Purging the P-900 pump, on page 116*.

NOTICE

Check valves have precision matched components and should only be disassembled further by a trained service engineer. If the problem cannot be corrected, the valve should be replaced completely.

9.4.5 Replacing valves IV-908 and INV-907H

Disassemble the valves and replace the distribution plate.

Note: *The spare parts are not interchangeable between the valve types. See the valve component manuals supplied with ÄKTA oligopilot plus for more details.*

9.5 Replacement of fuses

WARNING

Electrical shock hazard. All repairs should be done by service personnel authorized by Cytiva. Do not open any covers or replace parts unless specifically stated in the user documentation.

Note: *In the case of electrical failure, contact your Cytiva representative.*

9.6 Calibration

The table below lists the type and frequency of calibrations that can be done on the instrument. Refer to UNICORN user documentation and to the individual component User Manuals and Instructions for descriptions of how to perform these calibrations. The calibrations are performed from UNICORN by selecting ***System*** →***Calibrate*** in ***System Control***.

Component		How often
Pressure reading		When required.
Conductivity flow cell	Cell constant	Only necessary if specific conductivity with high accuracy is measured (***Cond_Calib***).
	Temperature	Must be done when changing the conductivity flow cell (***Temp***).
	Entering a new cell constant	Must be done when changing the conductivity flow cell (***Cond_Cell***).

9.7 Preparing ÄKTA oligopilot plus for standby

Follow the steps below if ÄKTA oligopilot plus should not be used for several weeks.

Step	Action
1	Disconnect the following bottles: • amidites • activator • oxidation reagent • capping reagent A • capping reagent B • thiolation reagent Cap all bottles tightly.
2	Store amidite solutions at 4°C and activator solution at room temperature.
3	Connect acetonitrile bottles to the amidite, activator and solvent lines.
4	Run the shutdown template method from ***UNICORN*** to flush the system with acetonitrile.

9.8 Storage

General recommendation

For storage, the system must first be cleaned as described in *Cleaning the system flow path, on page 111*.

Storage conditions

The following conditions shall be maintained while the system is in storage:

- Temperature: 2°C to 30°C (preferably room temperature)
- Relative humidity: 0% to 95%, non-condensing (preferably low humidity).

Before using the system after the storage, clean and sanitize the system, calibrate all monitors and perform a leakage test.

10 Troubleshooting

About this chapter

This chapter provides information to assist users and service personnel to identify and correct problems that may occur when operating the product.

If the suggested actions in this guide do not solve the problem, or if the problem is not covered by this guide, contact your Cytiva representative for advice.

In this chapter

10.1 UV curve problems

Error symptom	Possible cause	Corrective action
Noisy UV-signal, signal drift or instability	Poor UV fiber connections	Check the connections of the UV cell optical fiber. Replace if necessary.
	Dirty UV cell	Clean the UV cell by following the instructions in *Cleaning the Monitor UV-900 flow cell, on page 111*.
	Air in the pump or in the UV cell	Check that the flow restrictor FR-902 generates a back pressure of 2.0±1.0 bar at 10 mL/min.
	End line filters are clogged	Clean the end line filters.
The autozero level is higher than 1000 mAU at a wavelength of 280 to 290 nm.	The UV cell is clogged or dirty.	Clean the UV cell by following the instructions in *Cleaning the Monitor UV-900 flow cell, on page 111*.

10.2 Monitor problems

Monitor pH/C-900 and Monitor UV-900

Error symptom	Possible cause	Corrective action
No text on the front display	The power cable is not connected	Check that the power switch is in position ON.
	No power in the power outlet	Verify that the the power outlet works by connecting other equipment to it. If the power outlet is faulty, connect the power cable to a different power outlet.

10.3 Conductivity curve problems

Error symptom	Possible cause	Corrective action
Baseline drift or noisy signal	Air in the pump or the flow cell	Check the flow restrictor after the flow cell.
	Leaking tube connections	Tighten the connectors. If necessary, replace the connectors.
	Pump not working correctly	Troubleshoot the pump. Refer to *Section 10.4 Pump P-900, on page 142*.
	Dirty flow cell	Clean the flow cell according to the procedure in the *Monitor pH/C-900 Operating Instructions*, product code 29054925.
Conductivity measurement with the same buffer appears to decrease over time	Dirty flow cell	Clean the flow cell according to the procedure in the *Monitor pH/C-900 Operating Instructions*, product code 29054925.
Incorrect or unstable reading	Loose connection of conductivity flow cable	Check that the conductivity flow cell cable is connected properly.
	Incorrect pump and valves function	Check that the pump and valves operate correctly.

Error symptom	Possible cause	Corrective action
	Possible error in the COND cell	Test the COND monitor using any of the procedures below. **With KCl**: 1. Inject 10 to 20 mL of 1 mM KCl into the COND cell. The value in the display should be 150 ± 20 µS/cm. 2. Inject 10 to 20 mL of 2 mM KCl into the COND cell. The value in the display should be 280 ± 20 µS/cm. **With NaCL:** • Inject 10 to 20 mL of 2 mM NaCl into the COND cell. The value on the display should be 240 ± 20 µS/cm. Recalibrate if the above tests fail. If the problem persist after recalibration, contact a service representative or replace the COND cell.

10.4 Pump P-900

Important information

Note: *P-900 oligo pumps have a special piston seal made from a chemically resistant material and special check valves. Contact Cytiva if new parts are needed.*

Troubleshooting guide

Error symptom	Corrective action
No text on the front display	Check that the power supply cord is connected and the **Power** switch is in ON position **I**.
Liquid leaking between the pump head and the side panel	The piston seal or rinsing membrane is/are incorrectly fitted or worn. 1. Replace or re-install the seal or membrane. 2. Run-in carefully, see *Running-in a new piston seal, on page 123*.
Leaking connection and/or crystallised material around a connector	1. Unscrew the connector and check if it is worn or incorrectly fitted. If so replace the connector. 2. Gently tighten the connector with your fingers.
Erratic pump pressure	To check the pump function, make a recording of the pressure, or check the pressure in UNICORN. By observing the piston stroke indicator in the ***Check*** menu together with the pressure trace, the pump head which is functioning abnormally can be identified (see *Checking piston stroke* below). There can be several causes of an abnormal pressure recording, for example: • air trapped in the pump heads • partially blocked solvent filters • leaking connections • piston seal leakage • check valve malfunction • piston damaged. ***Note:*** *When changing the check valve, tighten to 10 Nm. This corresponds to about fingertight plus 1/8 of a turn.*

Error symptom	Corrective action
Irregular flow, very low flow	Probable causes: air or dirt in a check valve preventing it from closing to seal and hold the pressure. 1. Record the pressure (see *Erratic pump pressure* above). 2. Identify the faulty check valve by observing which pump head is delivering the flow (see *Checking piston stroke* below. 3. Try to clean the check valves in-place on the pump head by pumping 100% methanol for approximately 10 minutes. 4. If this does not correct the problem, follow the instructions for removing and then cleaning the valves. See *Section 9.4.4 Removing and cleaning the inlet and outlet check valves, on page 130*.

Checking piston stroke

To enable trouble shooting it is possible to check which pump module head that delivers flow:

Select main menu ***Check***, press **OK**.

The display shows the status of the pistons for both pump modules. ***A: Left*** means that the left pump head is delivering flow in the A pump and ***B: Right*** the right pump head in the B pump. At the changing point both are displayed. The A pump is closest to the front panel.

10.5 Tubing and connectors

Error symptom	Corrective action
External leakage	Check the tubing connections. Tighten or replace if required.
Internal leakage	Internal leakage can be detected at the small hole on the underside of the valve body. Internal valve parts may be worn. Change channel plate and distribution plate according to the relevant valve instruction.
High back-pressure (related to the tubings valves)	1. Perform cleaning-in-place according to the instructions in the relevant valve instruction. 2. Change channel plate and distribution plate according to the relevant valve instruction.

10.6 High system back pressure

Introduction

The system back pressure is high if the following is true:

- the pressure alarm sounds, which occurs at pressures higher than 20 bar
- the back pressure is over 15 bar and increasing.

Causes

High system back pressure can be caused by dirty column frits, blocked/bent tubing, a blocked UV flow cell, a blocked conductivity flow cell, or a blocked flow restrictor. High back pressure may also be generated from overloaded columns due to, for example, too high loading in combination with synthesis of long oligonucleotides and/or high bed heights.

Actions

If it is not possible to determine what causes the back pressure, abort the synthesis and follow the steps below.

Step	Action
1	In ***System Control*** →***Manual*** →***Flowpath***, select ***Bypass*** and click ***Execute***.
2	Select ***Pump*** and set ***Flow_A*** • to 5 mL/min for ÄKTA oligopilot plus 10 • to 50 mL/min, for ÄKTA oligopilot plus 100
3	Click ***Execute***.

10.7 IV-908 and INV-907-H valves

Error symptom	Possible cause	Corrective action
The valve does not switch.	The connection to the pump is not secure.	Check the connection between the valve and the pump for tightness.
	The connection to the pump is incorrect.	Check that the valve is connected to the **UniNet 1** socket.
	The number of the ID switch does not match in UNICORN.	Check that the ID switch setting in UNICORN matches the ID switch of the valve.
	The UniNet cable is faulty.	Replace the UniNet cable.
The valve switches to the wrong position.	The valve is incorrectly assembled after a replacement.	Check that the distribution plate marking **i/o** or **3** is horizontal. Remove and turn the distribution plate if required.
There is visible external leakage.	The tubing connections are not tight.	Tighten or replace the tubing connections as necessary.
There is internal leakage in the valve (visible at the small hole on the underside of the valve body).	Internal parts of the valve are worn.	Change the channel plate and distribution plate.
The back pressure is too high, either: • higher than 20 bar • 15 bars or higher, and rising	The valve is clogged.	Clean the valve.
	The channel plate or distribution plate is worn.	Change the channel plate and distribution plate.

10.8 Chemical problems

The following are common causes of chemical problems:

- There is too much (>30 ppm) water in the reagents. Use quality reagents and molecular sieves when needed.
- The reagents are too old. Amidites have a shelf life of 2-4 weeks, with G-amidite being more sensitive than others. T-amidite is the least sensitive.
- The amidite concentration is wrong. 5 gram and 10 gram amidite bottles are packed in the same type of bottle.
- Insufficient or no purging of amidites or solvents prior to the synthesis.
- Decrease in inert gas pressure caused by leaking bottle caps or tubing, or a bottle that was opened during synthesis.
- Flow fluctuations resulting from temporary gas pressure drops, causing cavitation gas bubbles to become trapped in one of the pump heads.

10.9 False trityl peaks

Error symptom	Possible cause	Corrective action
In the synthesis data view, the retention and duration values are unstable, or don't follow trends for different bases.	A false trityl peak has been detected.	Compare the retention and duration value for the calculated last efficiency. If these values differ significantly from the other values in the table, ignore the values for this peak.

10.10 No peak detected

First measures

If the message ***No Peak detected*** is displayed in UNICORN when ÄKTA oligopilot plus is in pause mode, follow the steps in the table below first.

Possible cause	Corrective action
One or more reagent bottles are empty.	Check and refill any empty bottles.
An amidite bottle is damaged.	Check the necks and seals of the amidite bottles. Replace all damaged bottles.
The amidite bottle seals are dirty.	Clean the amidite bottle seals with acetone.
There are leaks in the flow circuit.	Check the pumps, valves and the column for leaks.

Check if the orange detritylation peak elutes properly

Follow the steps below to check if the orange detritylation peak elutes properly.

Step	Action
1	Click ***Continue*** in ***System control***.
2	Check if the orange detritylation peak elutes during the following detritylation. • **If the color elutes:** Check the monitor occasionally and see if the error message recurs. • **If no color elutes:** The synthesis has failed. Restart the synthesis.

Check if the detritylation bottle is empty

Follow the steps below to check if the detritylation bottle is empty.

Step	Action
1	In ***System*** →***Control*** →***Manual*** →***Flowpath*** set ***Solvent_A*** to ***Detrit_3.7***. Click ***Execute***.
2	Connect a solvent resistant syringe to the purge valve on pump A.

Step	Action
3	Open the purge valve and draw solution into the syringe. When the solution enters the syringe, close the valve.
4	Move the syringe to the second purge valve on pump A.
5	Open the valve and draw solution into the syringe. When the solution enters the syringe, close the valve.
6	Press ***Continue*** and wait 10 seconds. Pump A should start; when it starts, click ***Hold***. Pump A continues pumping, but the method does not progress to the next break point.
7	When color from the detritylation starts to exit from the column, click ***Continue***. The synthesis continues by adding the next amidite. However, any negative effect of an empty detritylation bottle will not be revealed until the crude syntheses have been analysed (post-synthesis) for % full-length product and A260 yield.

Check if the amidite tubing is blocked

Run the troubleshooting method ***Delivery test***, or perform the steps below manually.

Step	Action
1	Replace the suspect amidite bottle with a 100 mL bottle filled with acetonitrile. If necessary, replace all amidite bottles.
2	Open ***System Control*** →***Manual*** →***Flowpath*** and select ***Amidite Acn_A/X***. Click ***Execute***.
3	Select ***Pump*** and set ***Flow_A*** to 10 mL/min. Wait for 2 minutes.
4	Select the suspected amidite position or go through the positions in order.
5	Observe the level in the bottles decreases. If the level is unchanged, install new PEEK tubing and clean the slider.

11 Reference information

About this chapter

This chapter lists the technical specifications of ÄKTA oligopilot plus. The chapter also includes a chemical resistance guide, ordering information, and Health and Safety Declaration forms for service.

In this chapter

11.1 Specifications

Introduction

This section lists the technical specifications of ÄKTA oligopilot plus.

In this section

11.1.1 ÄKTA oligopilot plus instrument

General technical specifications

Parameter	Value
Ingression protection	IP20
Supply voltage	100-240 V~
Maximum voltage fluctuation	± 10% from the nominal voltage
Frequency	50-60 Hz
Maximum power	600 VA
Transient overvoltages	Overvoltage category II
Fuse specification	T 6.3 AL 250 V
Dimensions (H × W × D)	610 × 450 × 480 mm
Weight	63 kg
Ambient temperature	4°C to 40°C
Relative humidity tolerance (non-condensing)	10% to 95%
Atmospheric pressure	840 to 1060 mbar (84 to 106 kPa)
Pollution degree	2
Acoustic noise level	70 dB A

11.1.2 ÄKTA oligopilot plus component materials

Introduction

This section lists the wetted materials of the components in ÄKTA oligopilot plus.

Abbreviations for composite materials

Abbreviation	Full name
CTFE	Chlorotrifluoroethylene
ETFE	Ethylenetetrafluoroethylene
FEP	Perfluoroethylenepropylene copolymer
FFKM	Perfluororubber
PE	Polyethylene
PEEK	Polyetheretherketone
PP	Polypropylene
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidenefluoride
Rulon™	Tetrafluoroethylene fluorocarbon

P-900 pump materials

Composite materials

- PE
- PEEK
- PTFE
- PVDF
- Rulon

Other materials

- Aluminium oxide
- Ruby/sapphire
- Stainless steel (Elgiloy)
- Titanium alloy

UV-900 monitor materials

Composite materials

- PEEK
- PTFE

Other materials

- Quartz
- Titanium alloy

pH/C-900 monitor materials

Composite materials

- CTFE
- FFKM
- PEEK
- PTFE

Other materials

- Glass
- Titanium alloy

UV/PV-908 valve materials

Composite materials

PEEK

INV-907 valve materials

Composite materials

PEEK

Flow restrictor materials

Composite materials

- ETFE
- PEEK
- PTFE

Other materials

Gold

Tubing materials

Composite materials

- ETFE
- PEEK
- PTFE

Other materials

FEP

Inlet filter materials

Composite materials

PP

Other materials

Titanium alloy

Unions and connectors materials

Composite materials

- ETFE
- PEEK

11.1.3 Specifications for P-900 pumps

Parameter	P-901	P-903
No. of pump modules	2	2
Pump head volume	100 mL	10 mL
Pressure range	0 to 100 bar	0 to 250 bar
Stroke volume	36 µL (ÄKTA oligopilot plus 10) 286 µL (ÄKTA oligopilot plus 100)	
Delay volume	< 600 µL (ÄKTA oligopilot plus 10) < 1200 µL (ÄKTA oligopilot plus 100)	

11.2 Chemical resistance

Chemical	Exposure < 1 day	Exposure up to 2 months	CAS no.	EEC no.	Comments
Acetonitrile	OK	OK	75-05-8	200-835-2	PP and PE swell.
Acetone, 10%	OK	Avoid			PVDF is affected by long term use.
Ammonia, 30%	OK	OK	7664-41-7	231-635-3	Silicone is affected by long-term use.
1-Butanol	OK	OK			
2-Butanol	OK	OK			
Chloroform	OK	Avoid			Kalrez™, CTFE, PP and PE are affected by long term use.
Cyclohexane	OK	OK			
Dimethyl sulph-oxide	Avoid	Avoid	67-68-5	200-664-3	PVDF is affected by long term use.
1, 4-Dioxane	Avoid	Avoid			ETFE, PP, PE and PVDF are affected by long term use.
Ethanol, 100%	OK	OK	75-08-1	200-837-3	
Hexane	OK	Avoid			Silicone not resistant. Pressure limit for PEEK decreases.
Hydrochloric acid, 0.1 M	OK	OK	7647-01-0	231-595-7	Silicone not resistant.
Hydrochloric acid, > 0.1 M	OK	Avoid			Silicone not resistant. Titanium is affected by long term use.
Isopropanol, 100%	OK	OK	67-63-0	200-661-7	
Methanol, 100%	OK	OK	74-93-1	200-659-6	

Chemical	Exposure < 1 day	Exposure up to 2 months	CAS no.	EEC no.	Comments
Nitric acid, diluted	OK	Avoid			Silicone not resistant.
Nitric acid, 30%	Avoid	Avoid			Elgiloy™ is affected by long term use.
Pyridine	OK	OK			ETFE, PP and PE not resistant.
Tetrahydrofuran	Avoid	Avoid			ETFE, CTFE, PP and PE are not resistant.
Toluene	OK	OK			Pressure limit for PEEK decreases.
Trichloroacetic acid, 1%	OK	OK	76-03-9	200-927-2	
Trifluoroacetic acid, 1%	OK	OK	176-05-1	200-929-3	

11.3 Recycling information

Introduction

This section contains information about the decommissioning of ÄKTA oligopilot plus.

Decontamination

The product must be decontaminated before decommissioning. All local regulations must be followed with regard to scrapping of the equipment.

Disposal of the product

When taking the product out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Recycling of hazardous substances

The product contains hazardous substances. Detailed information is available from your Cytiva representative.

Disposal of electrical components

Waste electrical and electronic equipment must not be disposed of as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of the equipment.

11.4 Regulatory information

Introduction

This section lists the regulations and standards that apply to the ÄKTA oligopilot plus instrument.

In this section

11.4.1 Contact information

Contact information for support

To find local contact information for support and sending troubleshooting reports, visit *cytiva.com/contact*.

Manufacturing information

The table below summarizes the required manufacturing information.

Requirement	Information
Name and address of manufacturer	Cytiva Sweden AB Björkgatan 30 SE 751 84 Uppsala Sweden
Telephone number of manufacturer	+ 46 771 400 600

11.4.2 European Union and European Economic Area

Introduction

This section describes regulatory information for the European Union and European Economic Area that applies to the equipment.

Conformity with EU Directives

See the EU Declaration of Conformity for the directives and regulations that apply for the CE marking.

If not included with the product, a copy of the EU Declaration of Conformity is available on request.

CE marking

The CE marking and the corresponding EU Declaration of Conformity is valid for the instrument when it is:

- used according to the *Operating Instructions* or user manuals, and
- used in the same state as it was delivered, except for alterations described in the *Operating Instructions* or user manuals.

11.4.3 Eurasian Economic Union Евразийский экономический союз

This section describes the information that applies to the product in the Eurasian Economic Union (the Russian Federation, the Republic of Armenia, the Republic of Belarus, the Republic of Kazakhstan, and the Kyrgyz Republic).

Introduction

This section provides information in accordance with the requirements of the Technical Regulations of the Customs Union and (or) the Eurasian Economic Union.

Введение

В данном разделе приведена информация согласно требованиям Технических регламентов Таможенного союза и (или) Евразийского экономического союза.

Manufacturer and importer information

The following table provides summary information about the manufacturer and importer, in accordance with the requirements of the Technical Regulations of the Customs Union and (or) the Eurasian Economic Union.

Requirement	Information
Name, address and telephone number of manufacturer	See *Manufacturing information*
Importer and/or company for obtaining information about importer	Cytiva RUS LLC 109004, Russian Federation Moscow Stanislavskogo str., 21, building 3, premises I, room 57 Telephone: +7 495 7877617 E-mail: *rucis@cytiva.com*

Информация о производителе и импортере

В следующей таблице приводится сводная информация о производителе и импортере, согласно требованиям Технических регламентов Таможенного союза и (или) Евразийского экономического союза.

Требование	Информация
Наименование, адрес и номер телефона производителя	См. *Информацию об изготовлении*
Импортер и/или лицо для получения информации об импортере	ООО "Цитива РУС" 109004, Российская Федерация город Москва, ул. Станиславского, д. 21, строение 3, помещение I, комната 57 Телефон: +7 495 7877617 Адрес электронной почты: *rucis@cytiva.com*

Description of symbol on the system label
Описание обозначения на этикетке системы

This Eurasian compliance mark indicates that the product is approved for use on the markets of the Member States of the Customs Union of the Eurasian Economic Union

Данный знак о Евразийском соответствии указывает, что изделие одобрено для использования на рынках государств-членов Таможенного союза Евразийского экономического союза

11.4.4 Regulations for North America

Introduction

This section describes the information that applies to the product in the USA and Canada.

FCC compliance

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Note: *The user is cautioned that any changes or modifications not expressly approved by Cytiva could void the user's authority to operate the equipment.*

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

11.4.5 Regulatory statements

Introduction

This section shows regulatory statements that apply to regional requirements.

EMC emission, CISPR 11: Group 1, Class A statement

NOTICE

This equipment is not intended for use in residential environments and may not provide adequate protection to radio reception in such environments.

South Korea

Regulatory information to comply with the Korean technical regulations.

NOTICE

Class A equipment (equipment for business use).

This equipment has been evaluated for its suitability for use in a business environment.

When used in a residential environment, there is a concern of radio interference.

주의사항

A급 기기 (업무용 방송통신 기자재)

이 기기는 업무용환경에서 사용할 목적으로 적합성평가를 받은 기기

로서 가정용 환경에서 사용하는 경우 전파간섭의 우려가 있습니다.

11.4.6 Declaration of Hazardous Substances (DoHS)

根据 SJ/T11364-2014《电子电气产品有害物质限制使用标识要求》特提供如下有关污染控制方面的信息。

The following product pollution control information is provided according to SJ/T11364-2014 Marking for Restriction of Hazardous Substances caused by electrical and electronic products.

电子信息产品污染控制标志说明
Explanation of Pollution Control Label

该标志表明本产品含有超过中国标准 GB/T 26572《电子电气产品中限用物质的限量要求》中限量的有害物质。标志中的数字为本产品的环保使用期，表明本产品在正常使用的条件下，有毒有害物质不会发生外泄或突变，用户使用本产品不会对环境造成严重污染或对其人身、财产造成严重损害的期限。单位为年。

为保证所申明的环保使用期限，应按产品手册中所规定的环境条件和方法进行正常使用，并严格遵守产品维修手册中规定的定期维修和保养要求。

产品中的消耗件和某些零部件可能有其单独的环保使用期限标志，并且其环保使用期限有可能比整个产品本身的环保使用期限短。应到期按产品维修程序更换那些消耗件和零部件，以保证所申明的整个产品的环保使用期限。

本产品在使用寿命结束时不可作为普通生活垃圾处理，应被单独收集妥善处理。

This symbol indicates the product contains hazardous materials in excess of the limits established by the Chinese standard GB/T 26572 Requirements of concentration limits for certain restricted substances in electrical and electronic products. The number in the symbol is the Environment-friendly Use Period (EFUP), which indicates the period during which the hazardous substances contained in electrical and electronic products will not leak or mutate under normal operating conditions so that the use of such electrical and electronic products will not result in any severe environmental pollution, any bodily injury or damage to any assets. The unit of the period is “Year”.

In order to maintain the declared EFUP, the product shall be operated normally according to the instructions and environmental conditions as defined in the product manual, and periodic maintenance schedules specified in Product Maintenance Procedures shall be followed strictly.

Consumables or certain parts may have their own label with an EFUP value less than the product. Periodic replacement of those consumables or parts to maintain the declared EFUP shall be done in accordance with the Product Maintenance Procedures.

This product must not be disposed of as unsorted municipal waste, and must be collected separately and handled properly after decommissioning.

有害物质的名称及含量
Name and Concentration of Hazardous Substances

产品中有害物质的名称及含量
Table of Hazardous Substances' Name and Concentration

部件名称 Compo-nent name	有害物质 Hazardous substance					
	铅 (Pb)	汞 (Hg)	镉 (Cd)	六价铬 (Cr(VI))	多溴联苯 (PBB)	多溴二苯醚 (PBDE)
18114042	X	0	0	0	0	0
18113679	X	0	0	0	0	0

0: 表示该有害物质在该部件所有均质材料中的含量均在 GB/T 26572 规定的限量要求以下。

X: 表示该有害物质至少在该部件的某一均质材料中的含量超出 GB/T 26572 规定的限量要求。

- 此表所列数据为发布时所能获得的最佳信息.

0: Indicates that this hazardous substance contained in all of the homogeneous materials for this part is below the limit requirement in GB/T 26572.

X: Indicates that this hazardous substance contained in at least one of the homogeneous materials used for this part is above the limit requirement in GB/T 26572

- Data listed in the table represents best information available at the time of publication.

11.4.7 Other regulations and standards

Introduction

This section describes the standards that apply to the product.

11.5 Ordering information

Contact details

For ordering information visit cytiva.com/oligo.

11.6 Health and Safety Declaration Form

On site service

cytiva

On Site Service Health & Safety Declaration Form

Service Ticket #:	

To make the mutual protection and safety of Cytiva service personnel and our customers, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts a repair. To avoid delays in the servicing of your equipment, complete this checklist and present it to the Service Engineer upon arrival. Equipment and/or work areas not sufficiently cleaned, accessible and safe for an engineer may lead to delays in servicing the equipment and could be subject to additional charges.

Yes	No	Review the actions below and answer "Yes" or "No". Provide explanation for any "No" answers in box below.
○	○	**Instrument has been cleaned of hazardous substances**. Rinse tubing or piping, wipe down scanner surfaces, or otherwise make sure removal of any dangerous residue. Make sure the area around the instrument is clean. If radioactivity has been used, perform a wipe test or other suitable survey.
○	○	**Adequate space and clearance is provided to allow safe access** for instrument service, repair or installation. In some cases this may require customer to move equipment from normal operating location prior to Cytiva arrival.
○	○	**Consumables, such as columns or gels, have been removed or isolated from the instrument and from any area that may impede access to the instrument.**
○	○	**All buffer / waste vessels are labeled. Excess containers have been removed from the area to provide access.**
Provide explanation for any "No" answers here:		

Equipment type / Product No:		Serial No:	

I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.

Name:		Company or institution:	
Position or job title:		Date (YYYY/MM/DD):	
Signed:			

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Product return or servicing

Health & Safety Declaration Form for Product Return or Servicing

Return authorization number:		*and/or* **Service Ticket/Request:**	

To make sure the mutual protection and safety of Cytiva personnel, our customers, transportation personnel and our environment, all equipment must be clean and free of any hazardous contaminants before shipping to Cytiva. To avoid delays in the processing of your equipment, complete this checklist and include it with your return.

1. Note that items will NOT be accepted for servicing or return without this form
2. Equipment which is not sufficiently cleaned prior to return to Cytiva may lead to delays in servicing the equipment and could be subject to additional charges
3. Visible contamination will be assumed hazardous and additional cleaning and decontamination charges will be applied

Yes	No	Specify if the equipment has been in contact with any of the following:	
◯	◯	Radioactivity (specify)	
◯	◯	Infectious or hazardous biological substances (specify)	
◯	◯	Other Hazardous Chemicals (specify)	

Equipment must be decontaminated prior to service / return. Provide a telephone number where Cytiva can contact you for additional information concerning the system / equipment.		
Telephone No:		
Liquid and/or gas in equipment is:	☐	**Water**
	☐	**Ethanol**
	☐	**None, empty**
	☐	**Argon, Helium, Nitrogen**
	☐	**Liquid Nitrogen**
	Other, specify	

Equipment type / Product No:		**Serial No:**	
I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.			
Name:		**Company or institution:**	
Position or job title:		**Date (YYYY/MM/DD)**	
Signed:			

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For local office contact information, visit cytiva.com/contact.
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To receive a return authorization number or service number, call local technical support or customer service.

Appendix A
Tubing

About this chapter

Numbers in the Figure reference column in *Tubing specifications for ÄKTA oligopilot plus 10, on page 175* and *Tubing specifications for ÄKTA oligopilot plus 100, on page 177* refer to the tubing numbers in the liquid flow path connection diagram, see *Appendix I Connection diagrams, on page 253*.

Tubing specifications for ÄKTA oligopilot plus 10

Figure ref.	Material, I.D. [mm], O.D.	Length [mm]	Tubing marker
K1	PEEK, 0.5, 1/16"	900	C (yellow)
K2	PEEK, 0.5, 1/16"	900	A (yellow)
K3	PEEK, 0.5, 1/16"	900	T (yellow)
K4	PEEK, 0.5, 1/16"	900	G (yellow)
K5	FEP, 2.9, 3/16"	290	
K6	FEP, 2.9, 3/16"	210	
K7	PEEK, 0.5, 1/16"	260	K7 (green)
K8	PEEK, 0.5, 1/16"	230	K8 (green)
K9	FEP, 2.9, 3/16"	290	
K10	FEP, 2.9, 3/16"	350	
K11	FEP, 2.9, 3/16"	350	
K12	FEP, 2.9, 3/16"	370	
K13	FEP, 2.9, 3/16"	225	
K14	FEP, 2.9, 3/16"	350	
K15	FEP, 2.9, 3/16"	250	
K17	FEP, 1.6, 1/8"	390	
K18	FEP, 1.6, 1/8"	455	

Figure ref.	Material, I.D. [mm], O.D.	Length [mm]	Tubing marker
K20	FEP, 1.6, 1/8"	245	
K21	FEP, 1.6, 1/8"	160	
K23	FEP, 1.6, 1/8"	255	
K24	PEEK, 0.5, 1/16"	410	K24 (blue)
K25[1]	PEEK, 0.5, 1/16"	820	K25 (green)
K26	PEEK, 0.5, 1/16"	320	K26 (blue)
K27	PEEK, 0.5, 1/16"	80	K27 (red)
K28	PEEK, 0.5, 1/16"	80	K28 (red)
K29	PEEK, 0.5, 1/16"	920	K29 (red)
K30	PEEK, 0.5, 1/16"	280	K30 (red)
K31	PEEK, 0.5, 1/16"	220	K31 (red)
K32	PEEK, 0.5, 1/16"	580	K32 (red)
K33	ETFE, 1.0, 1/16"	540	K33 (red)
K34	ETFE, 1.0, 1/16"	70	K34 (red)
K35	ETFE, 1.0, 1/16"	520	K35 (red)
K36	PEEK, 0.5, 1/16"	900	C* (yellow)
K37	PEEK, 0.5, 1/16"	900	A* (yellow)
K38	PEEK, 0.5, 1/16"	900	T* (yellow)
K39	PEEK, 0.5, 1/16"	900	G* (yellow)
K40	PEEK, 0.5, 1/16"	70	K40 (green)
K41	PEEK, 0.5, 1/16"	70	K41 (green)
K42	PEEK, 0.5, 1/16"	70	K42 (blue)
K43	PEEK, 0.5, 1/16"	70	K43 (blue)

[1] To function as a T-joint, the tubing and ferrule must both be level.

Tubing specifications for ÄKTA oligopilot plus 100

Figure ref.	Material, I.D. [mm], O.D.	Length [mm]	Tubing marker
K1	PEEK, 1.0, 1/16"	900	C (yellow)
K2	PEEK, 1.0, 1/16"	900	A (yellow)
K3	PEEK, 1.0, 1/16"	900	T (yellow)
K4	PEEK, 1.0, 1/16"	900	G (yellow)
K5	FEP, 2.9, 3/16"	290	
K6	FEP, 2.9, 3/16"	210	
K7	FEP, 1.6, 1/8"	240	K7 (green)
K8	FEP, 1.6, 1/8"	210	K8 (green)
K10	FEP, 2.9, 3/16"	350	
K11	FEP, 2.9, 3/16"	350	
K12	FEP, 2.9, 3/16"	370	
K13	FEP, 2.9, 3/16"	225	
K14	FEP, 2.9, 3/16"	350	
K15	FEP, 2.9, 3/16"	250	
K17	FEP, 2.9, 3/16"	390	
K18	FEP, 2.9, 3/16"	455	
K19	FEP, 2.9, 3/16"	200	
K20	FEP, 2.9, 3/16"	245	
K21	FEP, 2.9, 3/16"	160	
K23	FEP, 2.9, 3/16"	255	
K24	FEP, 1.6, 1/8"	360	K24 (blue)
K25	FEP, 1.6, 1/8"	900	K25 (green)
K26	FEP, 1.6, 1/8"	510	K26 (blue)
K27	ETFE, 1.0, 1/16"	80	K27 (red)
K28	ETFE, 1.0, 1/16"	80	K28 (red)
K29	FEP, 1.0, 1/16"	900	K29 (red)
K30	FEP, 1.0, 1/16"	270	K30 (red)

Figure ref.	Material, I.D. [mm], O.D.	Length [mm]	Tubing marker
K31	FEP, 1.6, 1/8″	200	K31 (red)
K32	FEP, 1.6, 1/8″	500	K32 (red)
K33	ETFE, 1.0, 1/16″	540	K33 (red)
K34	ETFE, 1.0, 1/16″	70	K34 (red)
K35	ETFE, 1.0, 1/16″	520	K35 (red)
K36	PEEK, 1.0, 1/16″	900	C* (yellow)
K37	PEEK, 1.0, 1/16″	900	A* (yellow)
K38	PEEK, 1.0, 1/16″	900	T* (yellow)
K39	PEEK, 1.0, 1/16″	900	G* (yellow)
K40	FEP, 1.6, 1/8″	70	K40 (green)
K41	FEP, 1.6, 1/8″	70	K41 (green)
K42	FEP, 1.6, 1/8″	70	K42 (blue)
K43	FEP, 1.6, 1/8″	70	K43 (blue)

Appendix B
Oligonucleotide synthesis chemistry

About this chapter

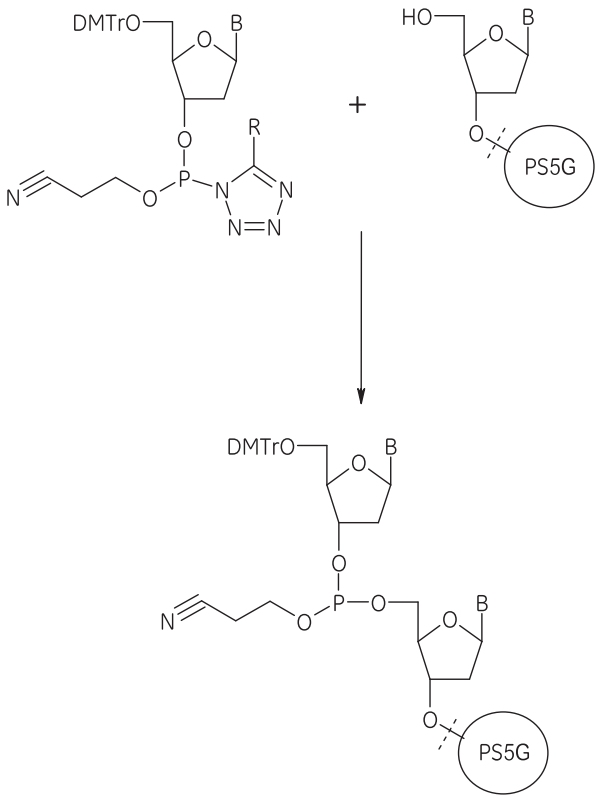
This chapter gives a general introduction to the chemistry used in solid-phase synthesis of oligonucleotide using ÄKTA oligopilot plus. For more detailed information, refer to literature at cytiva.com/oligo.

In this chapter

B.1 The coupling reaction

Overview

Synthesis of oligonucleotides involves sequential addition of amidite monomers, see the image below. This occurs via spontaneous coupling reactions between the 3'-phosphite group of the monomer in solution (left) and the 5'-hydroxyl group of the monomer attached to the solid support.



Note: *Synthesis chemistry is essentially the same for DNA and RNA oligonucleotides, with the exception of the molecular structure and protecting group scheme of the amidite reagents.*

The coupling reaction in ÄKTA oligopilot plus

Coupling is performed in ÄKTA oligopilot plus using dry acetonitrile as a solvent. Water hydroxyl groups compete strongly with the sugar hydroxyl groups. Thus, anhydrous conditions are essential for efficient coupling.

Many batch synthesis protocols use a large excess of monomers to overcome the competitive effect of traces of water in the solvents. ÄKTA oligopilot plus methods are designed to use a minimum of monomers, which makes completely anhydrous conditions essential for successful synthesis.

Tip: *Protected nucleotides as phosphoramidites should be used in the coupling reaction, since they are highly soluble in anhydrous organic solvents such as acetonitrile. Using unprotected nucleotide phosphoramidites can lead to ambiguous side reactions.*

Calculating the coupling efficiency

The coupling efficiency is calculated by integrating the area under the detritylation peak. Either UV or conductivity can be used for the calculation.

Note: *The coupling efficiencies are calculated using same bases, (i.e., a dA with previous dA). Therefore, the calculated efficiency is always 100% after the first calculation. This affects the calculated average coupling efficiency for the synthesis.*

Efficiency formula

$$efficiency = (Area(n)/Area(n'))^{1/b} \qquad [\%]$$

b = the number of bases since last identical base given by *Base_id*

If the coupling efficiency falls below a pre-set value, the synthesis can be set to pause to allow the user to continue or not. This setting is made in UNICORN System Control.

B.2 Protection chemistry

Principles of chemical protection

Certain molecular groups are used to prevent side reactions during synthesis. The protection groups are either **permanent** or **temporary**. The differences are outlined in the following table.

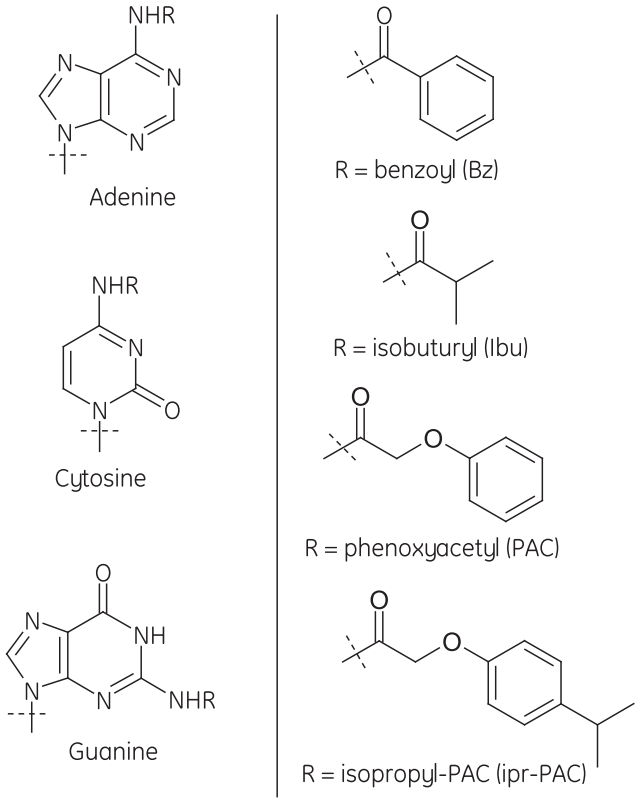
Types of chemical protection

	Permanent protection	Temporary protection
Removal of protection groups	After a complete synthesis	After a step in the synthesis
Required on	Primary amine groups of all heterocyclic bases • Prevents nucleophilic reactions with the amine group	5'-hydroxyl group of the incoming nucleotide • Avoids coupling of several nucleotides in a step
	One hydroxyl group in the phosphate residue • Prevents formation of multiple bonds to the phosphate group	

Other functional groups in the nucleotides do not react under the synthesis conditions and therefore do not require protection.

Amine protection (permanent)

Primary amine groups on the heterocyclic bases are generally protected as amides as in the following image.



Strategies in DNA amidite protection

For DNA amidite protection, two strategies are available: **standard protection** and **PAC protection**. The details are outlined in the following table.

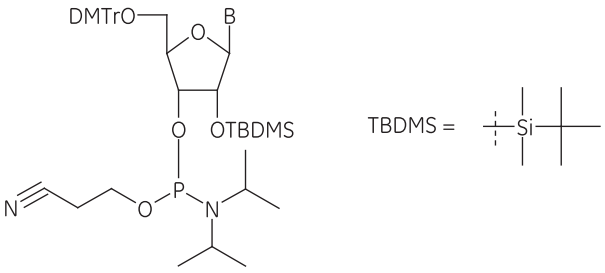
Base	Standard protection	PAC protection
A	Benzoyl	Phenoxyacetyl (PAC)
C	Benzoyl	Isobuturyl
G	Isobuturyl	Isopropylphenoxyacetyl (ipr-PAC)
T	*None*	*None*

RNA amidite protection

In RNA amidite protection, the fast protecting groups in the following table are typically used.

Base	Protection
A	Phenoxyacetyl (PAC)
C	Acetyl (Ac)
G	Isopropylphenoxyacetyl (ipr-PAC)
U	*None*

In addition, RNA amidites have a protecting t-butyldimethylsilyl (TBDMS) group on the 2' sugar hydroxyl.



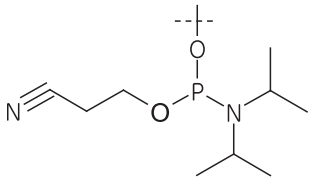
Deprotection of phosphoramidites

Deprotection of the amine protecting groups of phosphoramidites require treatment with concentrated ammonium hydroxide overnight at 55 to 60ºC.

Phosphate protection (permanent)

Several methods are available for protecting the phosphite hydroxyl group, according to the coupling method used for generating oligonucleotides.

The method used in ÄKTA oligopilot plus is to protect the hydroxyl group with a ß-cyanoethyl group. This prevents alkylation of thymine during synthesis, which may occur if a methyl protecting group is used.



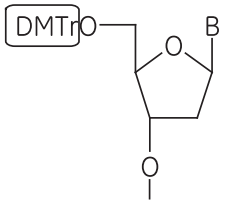
Phosphate deprotection

The recommended method for removing the ß-cyanoethyl group from the phosphate backbone is by treatment with 20% diethylamine (DEA) in ACN.

Phosphate deprotection may also be performed with concentrated ammonium hydroxide. However, this could result in modifications of the heterocyclic bases.

Sugar hydroxyl protection (temporary)

The 5'-hydroxyl group on the incoming nucleotide is protected with a 4,4'-dimethoxytrityl (DMTr) group as the following image shows.



The acid-labile DMTr group is stable under the controlled conditions of coupling. The absorbance or conductivity is measured when the DMTr is cleaved to give a measure of the coupling efficiency.

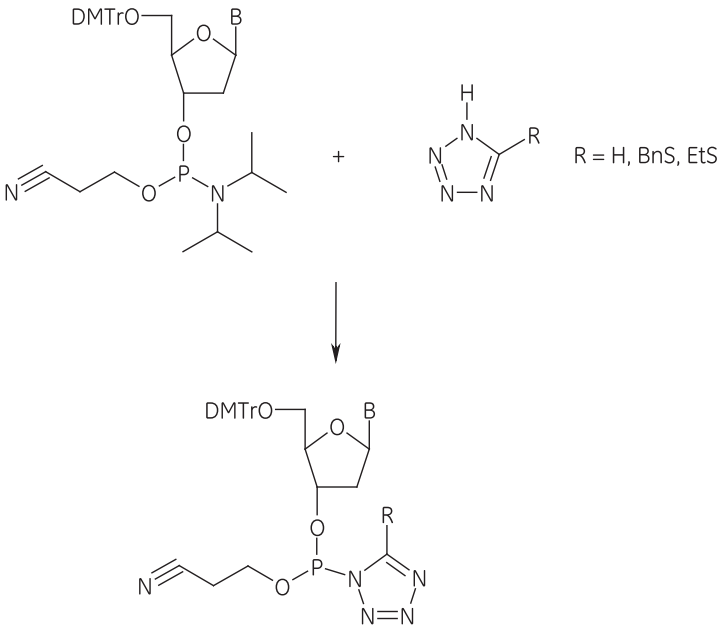
Sugar hydroxyl deprotection

The sugar hydroxyl group is removed in the beginning of each cycle in the synthesis by treatment with 3% dichloroacetic acid (DCA) in toluene.

B.3 Activation chemistry

Overview

Amidites as supplied have a secondary amine attached to the phosphorus moiety, which is activated under weakly acidic conditions, converting the amidite into a tetrazolide derivative (see image below).



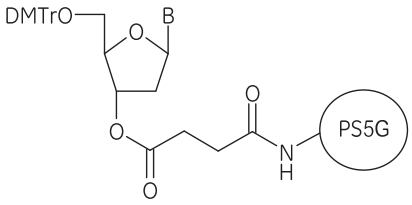
The amidite conversion occurs as the amidite is added together with an activator to the solid support.

B.4 The solid support

Overview

Oligonucleotide synthesis in ÄKTA oligopilot plus takes place on a solid support consisting of cross-linked polystyrene beads. Reagents in solution are passed through a column packed with the support, and the growing oligonucleotide remains attached to the support throughout the synthesis.

The support is primed with a nucleoside attached to the end of a linker as in the following image. The linker is then cleaved after the synthesis is complete. This nucleoside becomes the 3'-terminal residue in the final product.



Standard and customized Primer Supports 5G loaded with various nucleosides at different loadings are available for use in both DNA and RNA synthesis. More information is available at cytiva.com/oligo.

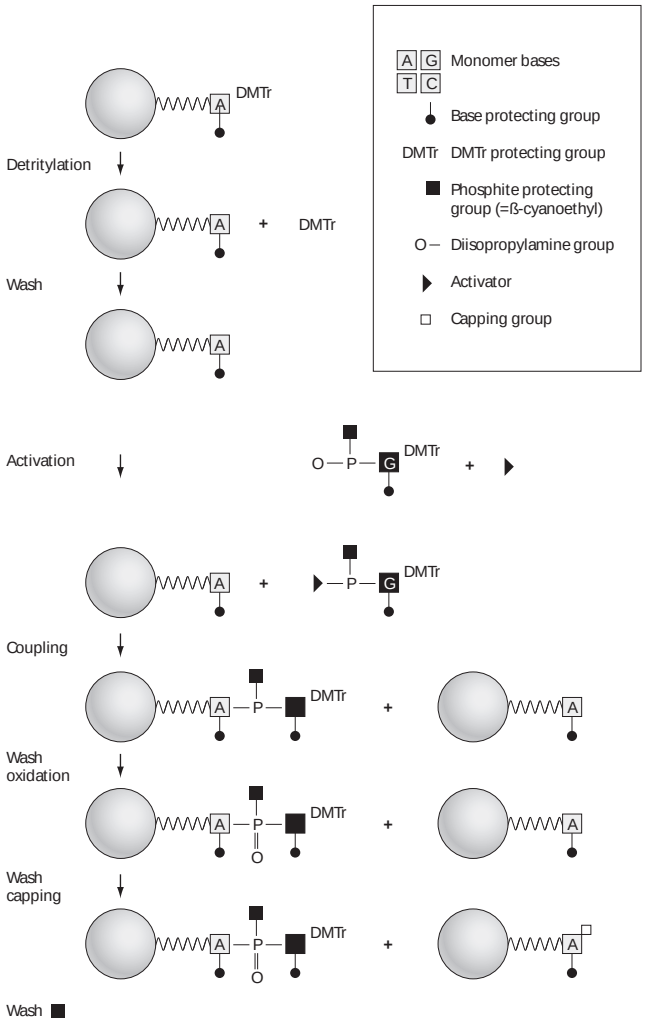
B.5 The synthesis cycle

Overview

The synthesis cycle adds one residue to the growing oligonucleotide on the solid support and consists of four major steps:

- Detritylation
- Coupling
- Oxidation
- Capping

In addition, there are several washing steps to ensure that no residual reagent from previous steps interfere with the next reaction step.



Stage	Description
Detri-tyla-tion	The 5'-DMTr group is removed from the terminal residue on the solid support. • The hydroxyl group is available for coupling
Coupli ng	The incoming amidite is activated with an activator. This forms a 3'-5' phosphite link to the growing oligonucleotide. ***Note:*** *Completely anhydrous conditions are essential for the efficiency of this step.*

Stage	Description
Oxidation	The phosphite triester is oxidated to a phosphotriester using iodine/water in pyridine. ***Note:*** *If phosphorothioate linkages are desired at this stage, use a thiolation reagent instead of iodine/water as the oxidising reagent.*
Capping	Unreacted 5'-hydroxyl groups are converted to acylates, which prevents the formation of heterogeneous oligonucleotide products.

Detritylation

Before detritylation, the support is washed with acetonitrile to remove reagents from the previous step. After detritylation, the support is washed again with acetonitrile.

Detritylation is performed using 3% dichloroacetic acid (DCA) in toluene. Depending on the scale, treatment for 2 to 5 minutes is sufficient for this reaction to occur, although the amount of detritylation reagent is more important than the time.

When the DMTr group is released it is monitored by UV light to measure the efficiency of coupling. The amount of DMTr released is directly correlated with the amount of full-length oligonucleotide on the solid support (i.e. the amount of the last nucleotide added).

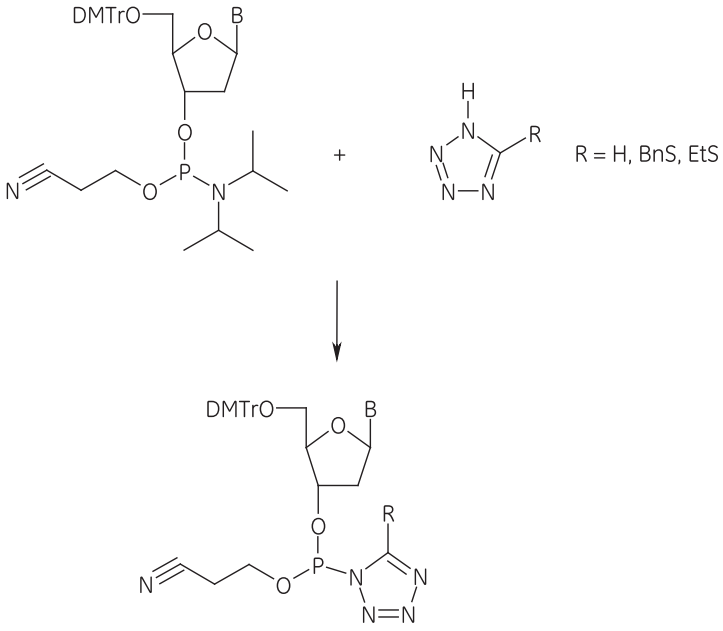
Note: *Harsh acid treatment can lead to depurination of adenosine with resultant breakage of the oligonucleotide chain when the final product is deprotected. Since the breakage does not occur until after synthesis is complete, it is not detected by DMTr monitoring.*

Choose detritylation conditions which minimize depurination of standard A-amidite.

Coupling

Coupling takes place when a mixture of amidite and an activator in dry acetonitrile is passed through the solid support. The activator used is an activated amidite, referred to as tetrazolide derivative.

The amidite is converted by reaction with the activator into a tetrazolide derivative containing a highly reactive phosphite group, se image below.



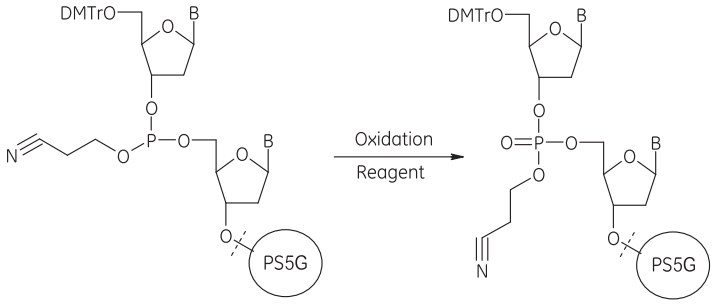
This intermediate reacts readily with any electron-donor: under the conditions of synthesis, the only donor available is the 5'-hydroxyl group on the growing oligonucleotide. Within a few minutes the coupling reaction efficiency is typically ≥99% for DNA and ≥98.5% for RNA.

By maintaining strictly anhydrous conditions and reagent quality, the need for a large excess of amidites is avoided. Synthesis in ÄKTA oligopilot plus typically obtains better than 98.5% coupling efficiency for DNA with a 1.5 molar excess of amidite, provided that the reagents are of the highest quality and that solvents are completely anhydrous. By far, the most common cause of failure in oligonucleotide synthesis is the use of poor quality or wrongly formulated reagents.

Oxidation

Before oxidation, the support is washed with acetonitrile to remove activator and unreacted amidite.

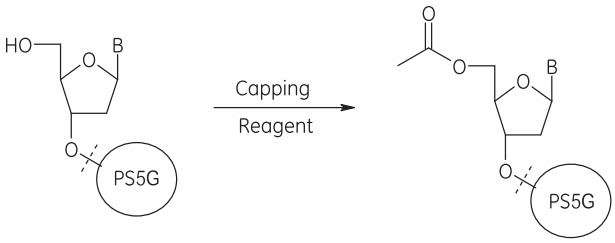
The phosphite group is oxidized to a more stable phosphate, using a solution of 0.05 M iodine in 9:1 pyridine/water, see the following image.



Capping

In this step, any unreacted 5'-hydroxyl groups on the oligonucleotide are capped. This ensures that only complete chains will have a nucleotide added in subsequent coupling steps, and helps to ensure a homogeneous product.

Capping involves acylation using acetic anhydride in a mixture of N-methylimidazole, collidine and acetonitrile. Other acylation reagents may also be used. The capping reagent is prepared in ÄKTA oligopilot plus by mixing capping solution A (20% N-methylimidazole in acetonitrile) and capping solution B (20% acetic anhydride and 30% sym-collidine in acetonitrile). The reaction scheme is shown in the following image.



After washing with acetonitrile to remove excess capping reagent, the oligonucleotide is ready for the next cycle or for post-synthetic processing.

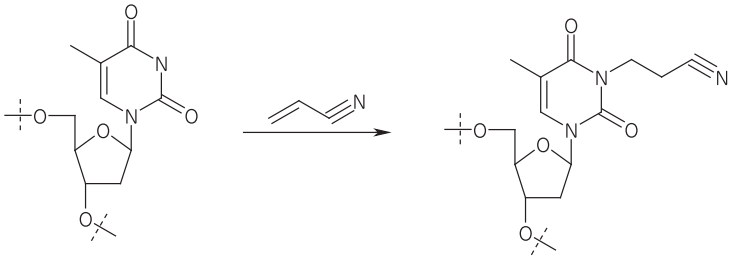
B.6 Post-synthetic processing

Overview

After the synthesis is complete, the column with the support bound oligonucleotide is removed from the system and processed further for cleavage and deprotection. These procedures are described in the operating instructions for ÄKTA oligopilot plus.

Side reactions when removing protecting groups

Removal of β-cyanoethyl phosphate protecting groups from oligonucleotides in concentrated ammonium hydroxide solutions at temperatures ranging from 26 to 650°C could result in modifications of the heterocyclic bases, especially thymine and also cytosine.



The β-cyanoethyl group from the phosphate back bone is eliminated in ammonium hydroxide by a β-elimination mechanism and forms acrylonitrile which is the reactive species in alkylating and modifying the bases.

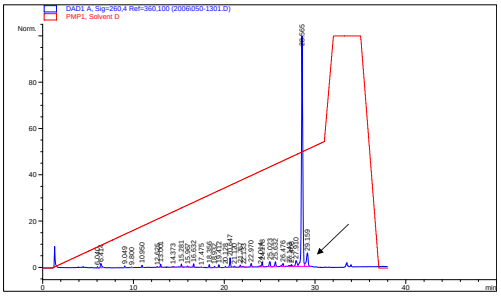
What DEA treatment does

The β-elimination is suppressed if the oligonucleotides (while still attached to the solid support) are treated with a 20% solution of diethylamine (DEA) in anhydrous acetonitrile through the column for 10 minutes, followed by regular cleavage and deprotection in ammonium hydroxide solution. This modified deprotection protocol increases the yield of the full-length oligonucleotide product by 3 to 7%, depending on the composition of the oligonucleotide.

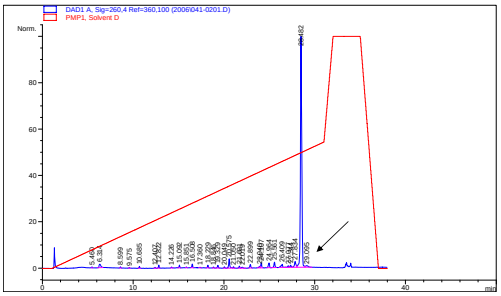
Effects of DEA treatment

The following diagrams shows the differences resulting from DEA treatment. Note the differences in peak heights at the arrows.

Without DEA treatment



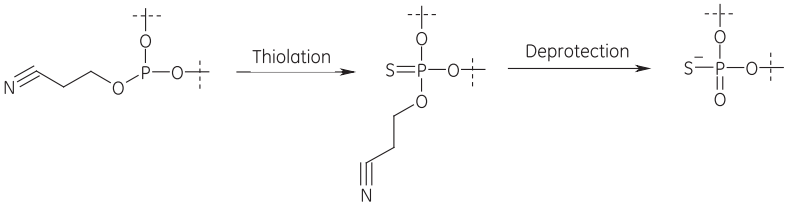
DEA treated oligonucleotide



B.7 Thiolated oligonucleotides

Overview

Thiolation reagents are compounds that performs thiolation of phosphite triesters, creating phosphorothioate bonds in the oligonucleotide sequence.

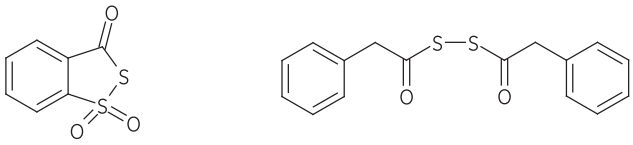


Oligonucleotides modified in this way are less susceptible to nuclease digestion than standard oligonucleotides, and have been used successfully as antisense sequences to inhibit gene expression by binding stably to the sense strand of target sequences.

To make such a change in the synthesis on ÄKTA oligopilot plus, the sequence should be modified in UNICORN. Thiolation reagent will then be introduced automatically during the oxidation step of synthesis.

Example reagents

Common thiolation reagents are Beaucage (left) and PADS (right).



Appendix C

Oligonucleotide analysis and purification

About this chapter

This chapter describes methods used for analysis and purification of oligonucleotides.

Ion exchange analysis

Ion exchange is used for analyzing both phosphodiester and phosphorothioate oligonucleotides. This technique is very efficient in detecting failure sequences as small as single nucleosides and dinucleotides. The single nuceloside usually elute as first peak in the chromatogram, next to the flow through peak and is usually very small. Peak sizes larger than 5 % are indicative of problems during the first synthesis cycle. Common problems are lack of purge, no column wash or wet reagents.

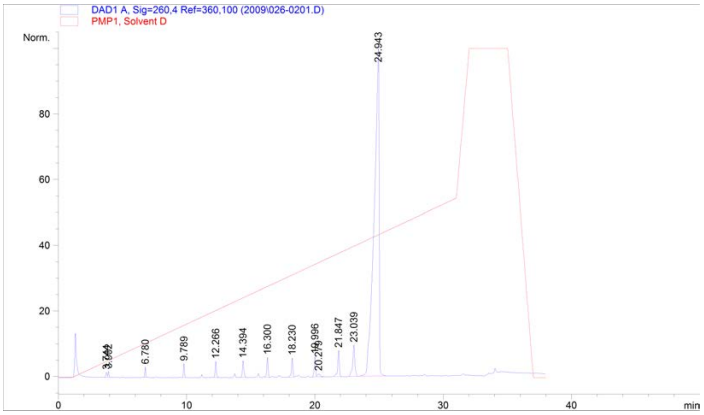
Recommended analysis conditions

	Phosphorodiester oligonucleotides	Phosphorothioate oligonucleotides
Column	NucleoPac PA 100 4x250 mm, DIONEX, p/n 43010	
Column temperature	+50°C	
Injection volume	2 µL	
Flow rate	1.0 mL/min	
Eluent A	10 mM Tris, 10 mM $NaClO_4$	1 mM EDTA, 25 mM TRIS, 10% acetonitrile, pH 8.0
Eluent B	10 mM Tris, 300 mM $NaClO_4$	1 mM EDTA, 25 mM TRIS, 10% acetonitrile, 3 M NH_4Cl, pH 8.0
Gradient	1 to 55% in 30 min	1 to 70%B in 40 min

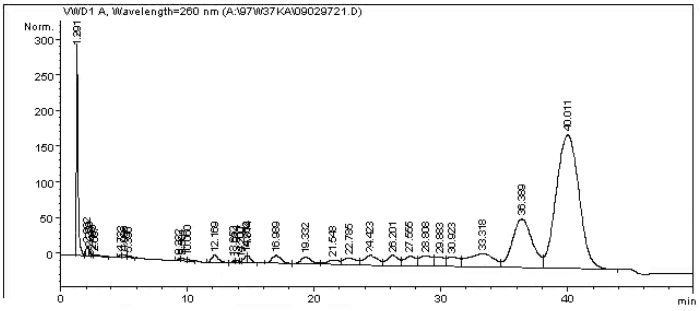
	Phosphorodiester oligo-nucleotides	Phosphorothioate oligonucleo-tides
Other	• Sample concentration: 10 OD/mL	1. Flow rate: 1.0 mL/min 2. UV: A260 3. Sample: 20 AU/(260nm)/mL, Trityl-ON

Example chromatograms

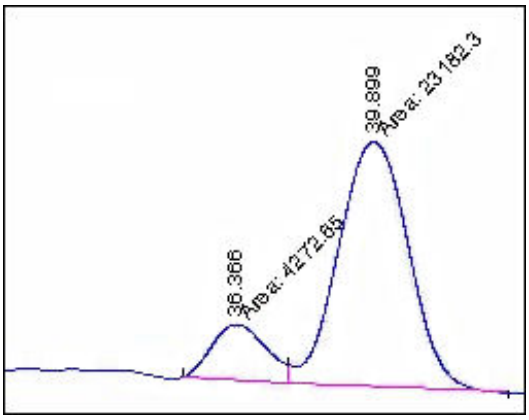
Phosphodiester nucleotides



Phosphorothioate nucleotides



Calculating the PO value of the 20-mer



$$\frac{area1}{area1+area2} = \frac{4272.65}{4272.65+23182.3} = 0.155$$

$$1 - \sqrt[19]{1-0.155} = 0.0088 = 0.88\%$$

RPC analysis

Reversed phase chromatography (RPC) analysis is an excellent technique for separating Trityl ON and Trityl OFF products. This method can also be used to see if the oligonucleotide is fully deprotected. If the deprotection is incomplete, the main peak will be divided into a number of peaks, depending on how many protecting groups the oligonucleotide still has on the bases.

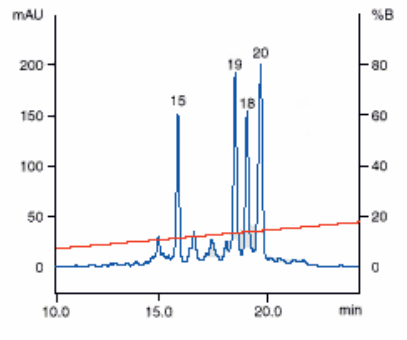
The RPC column from Cytiva, SOURCETM 5RPC ST 4.6/150, gives very good resolution even without the trityl group, since the column packing is polymer based and can be run at high pH.

Recommended analysis conditions

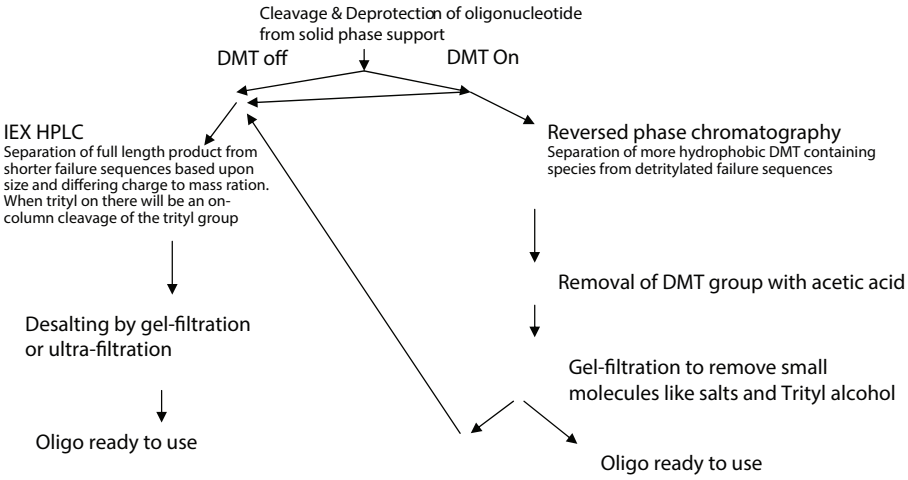
Parameter	Value
Column	SOURCE 5RPC ST 4.6/150
Eluent A, pH = 12	• 10 mM NaOH • 0.1% tetraethylammonium chloride • 1% acetonitrile
Eluent B, pH = 12	50% acetonitrile
Gradient	0% to 40%
Flow rate	1 mL/min

Recommended analysis conditions

The chromatogram below contains a mix of oligonucleotides with 15, 18, 19 and 20 bases.



Scheme for oligonucleotide purification



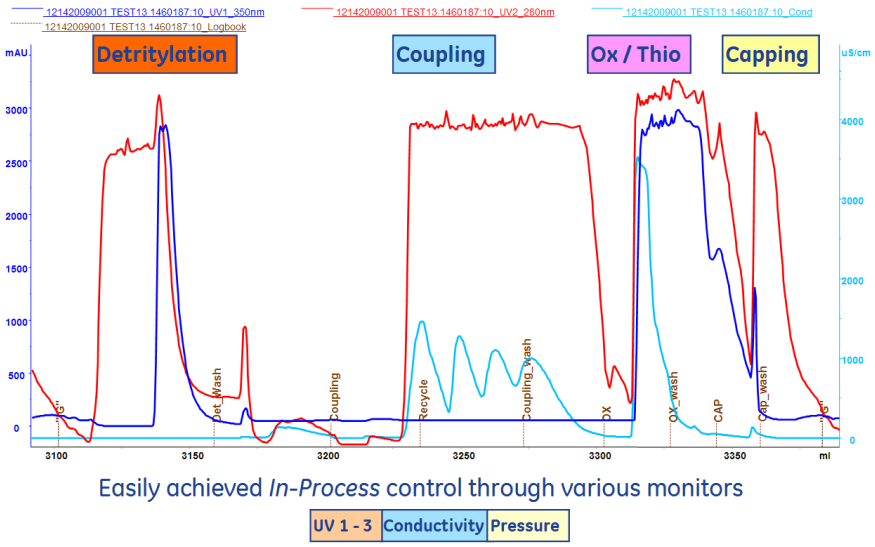
Appendix D
Synthesis flow charts

About this chapter

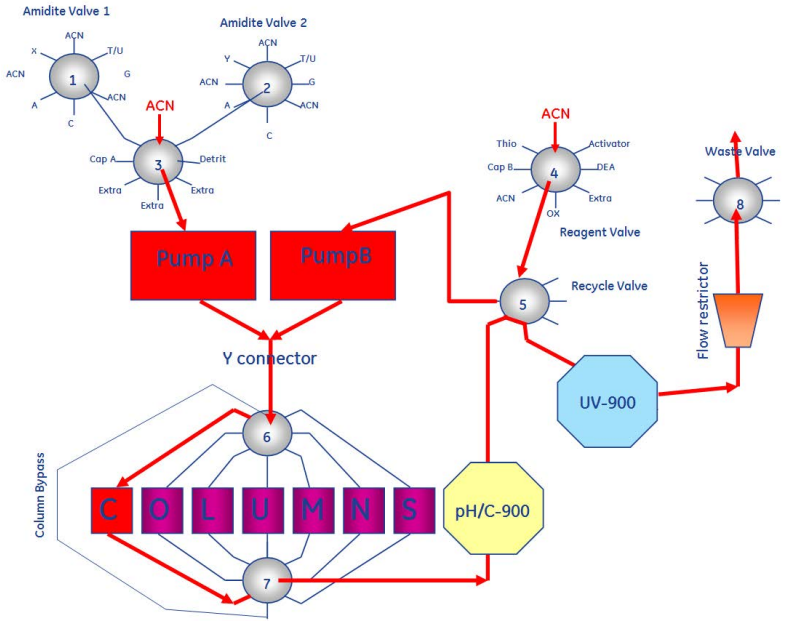
This chapter contains graphs of the stages of the synthesis cycle.

In this chapter

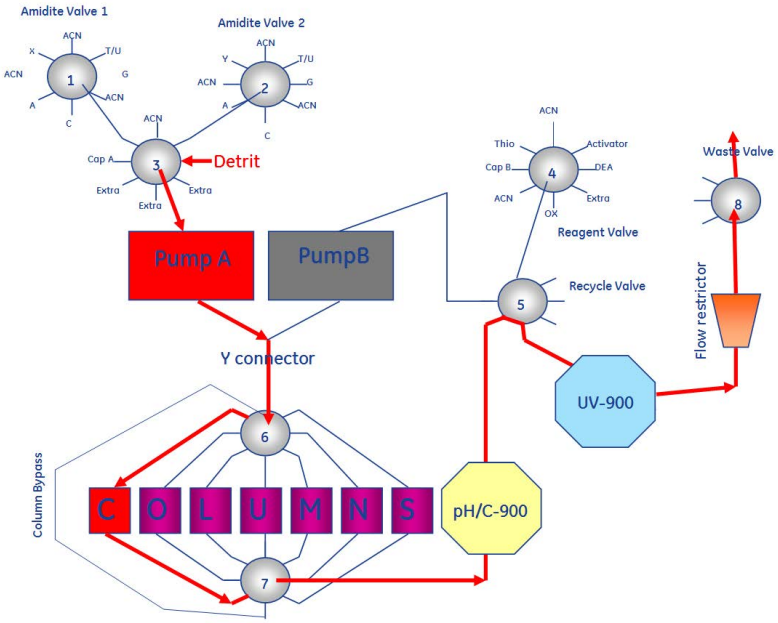
D.1 Synthesis cycle overview



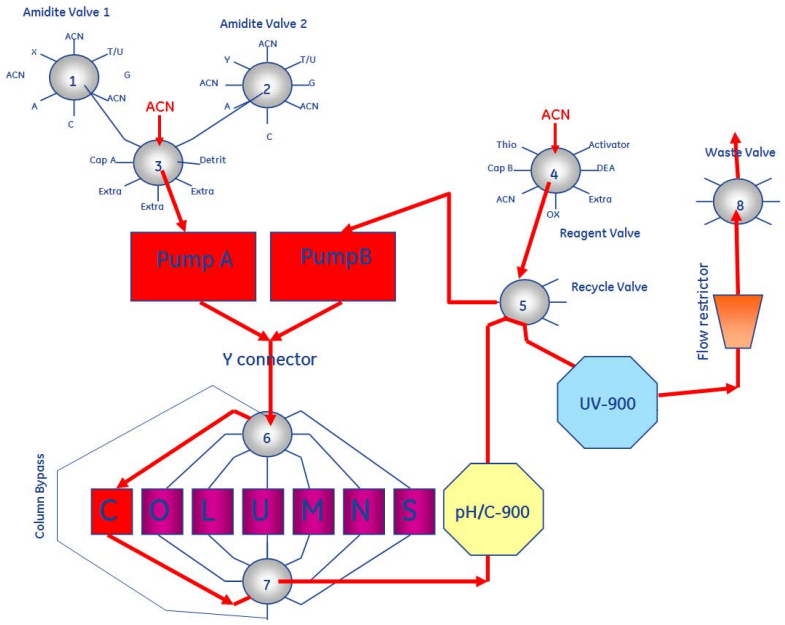
D.2 Column wash



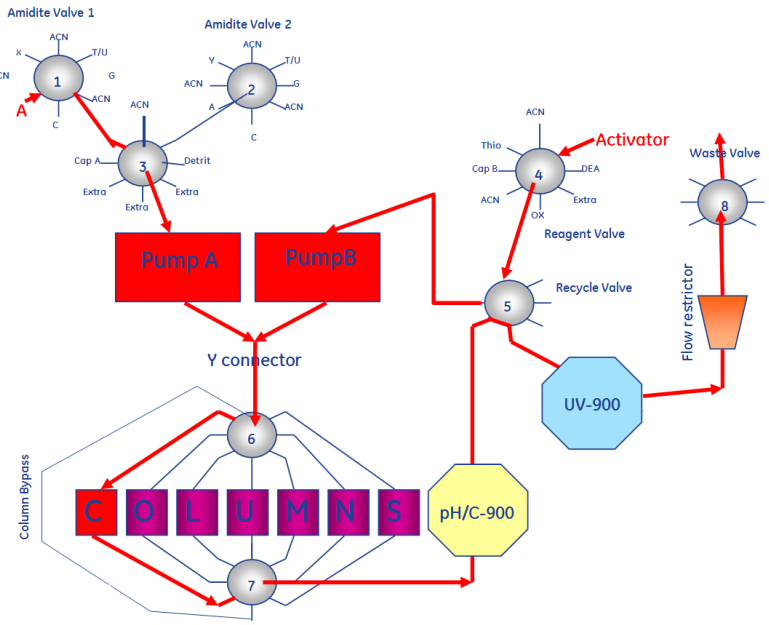
D.3 Detritylation



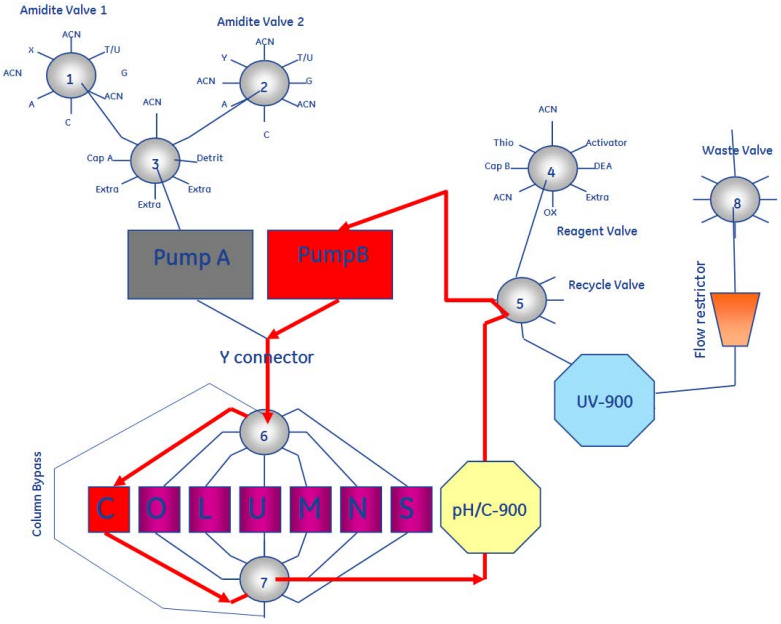
D.4 Detrit wash



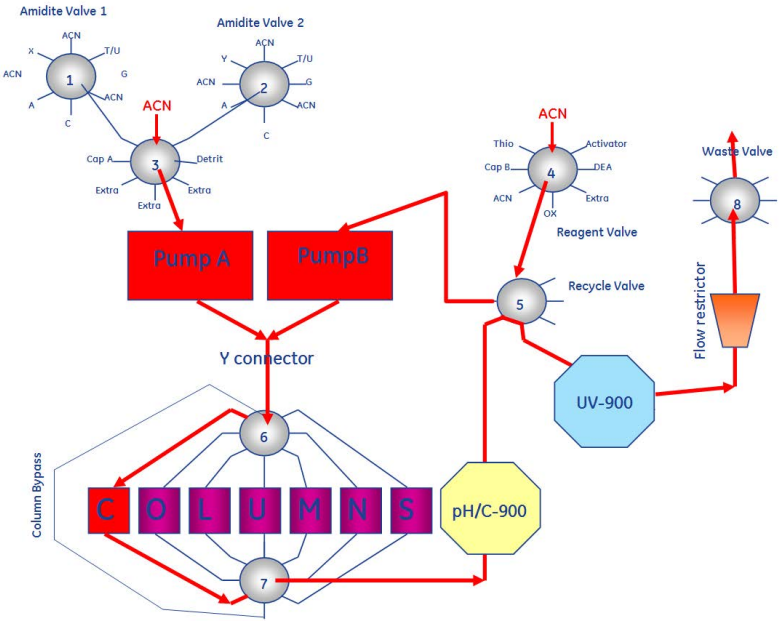
D.5 Adding coupling reagents



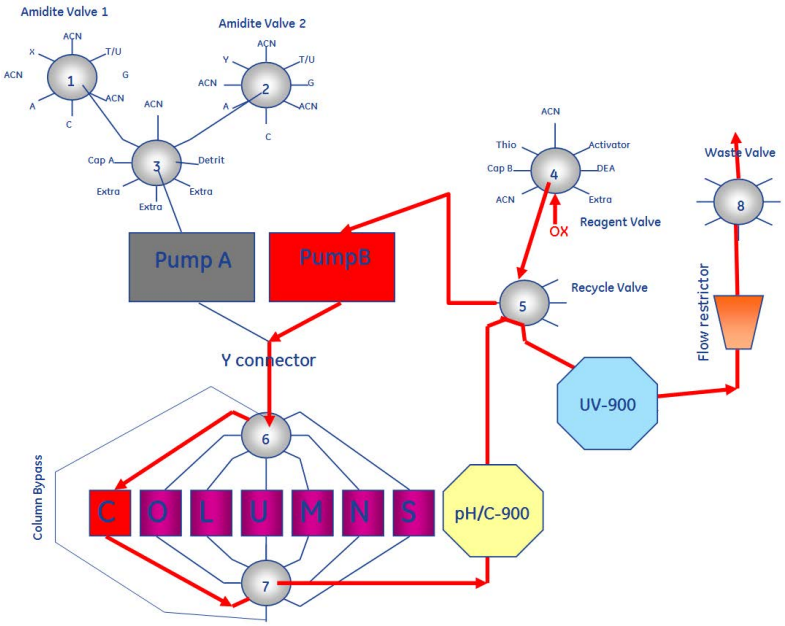
D.6 Recirculation of coupling reagents



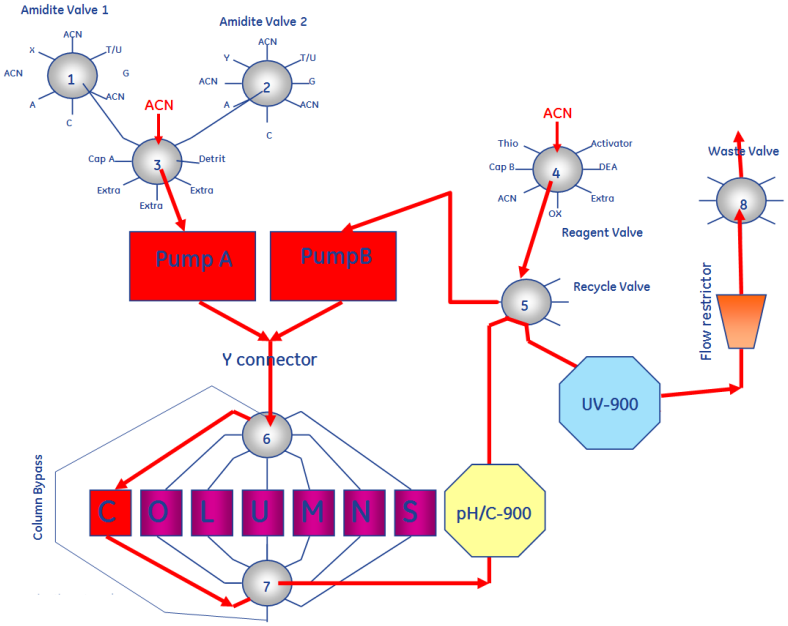
D.7 Coupling wash



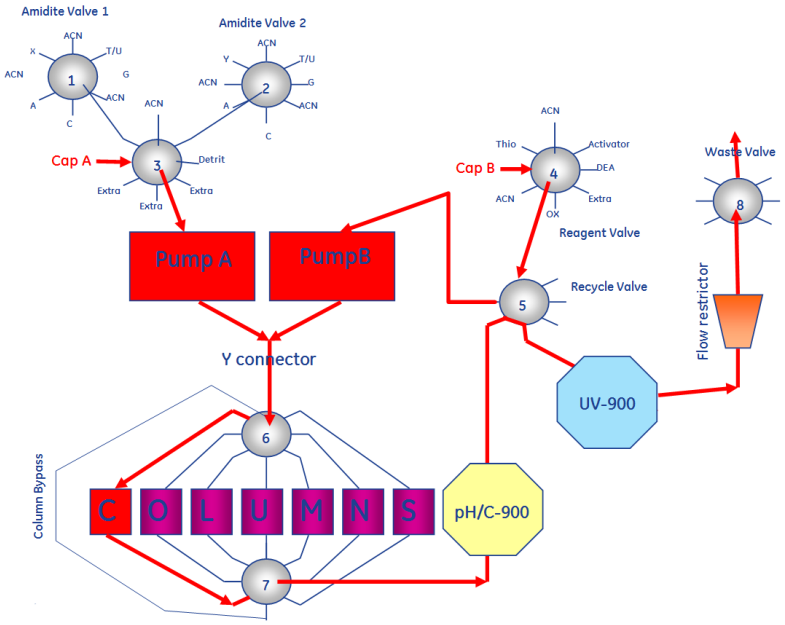
D.8 Oxidation



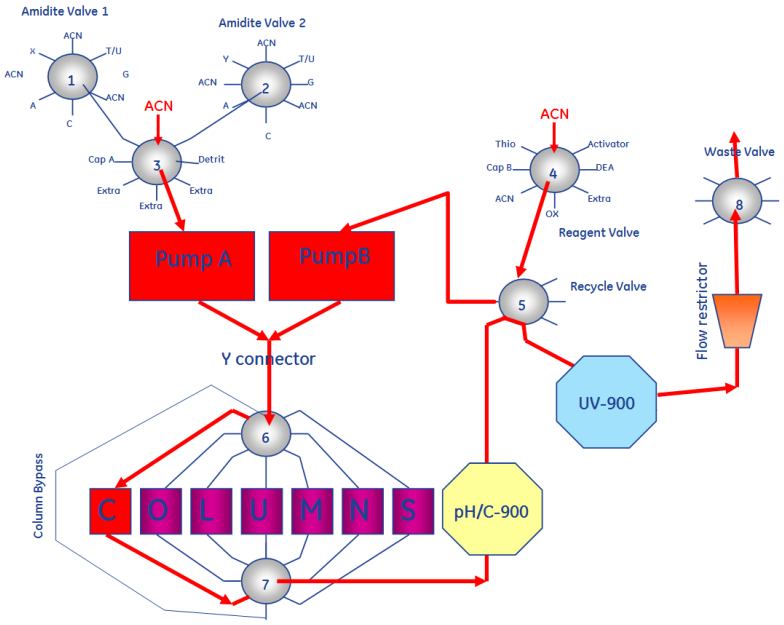
D.9 Oxidation wash



D.10 Capping


Amidite Valve 1
Amidite Valve 2
ACN
T/U
G
ACN
A
C
X
Y
1
2
3
Cap A
Detrit
Extra
Extra
Extra
Pump A
PumpB
Y connector
Cap B
Thio
Activator
DEA
OX
4
Reagent Valve
Recycle Valve
5
Waste Valve
8
Flow restrictor
UV-900
6
7
Column Bypass
C
O
L
U
M
N
S
pH/C-900

D.11 Capping wash



Appendix E

Method description for ÄKTA oligopilot plus

About this chapter

This chapter contains a method description for ÄKTA oligopilot plus. The method description is valid for ÄKTA oligopilot plus 100, with comments for ÄKTA oligopilot plus 10.

Subroutines

In the variable list, all synthesis parameters can be programmed as long as the general structure of the cycle is based on the following order of subroutines:

- Detritylation
- Detrit wash
- Coupling
- Oxidation/thiolation
- Capping

Note: *Do not set two pump instructions on the same breakpoint (time, volume, or column volume). Only the first instruction will be executed, which could have serious consequences to the synthesis.*

Setting method blocks on the same breakpoint prevents this problem.

Note: *Method blocks having bases that is either volume or column volume (CV) must to contain a flow instruction in breakpoint zero AND between all increasing breakpoints in a block. Otherwise, the method run will stop, since UNICORN cannot calculate volumes to proceed if the flowrate is zero.*

Terminology

equivalent

Molar equivalence of either amidites or iodine (I_2).

breakpoint

Point in a method block at which an instruction is issued. The first instruction is always issued at breakpoint 0.

contact time

Minimum time that every part of the column has contact with a specific reagent. The contact time is calculated after one equivalent is consumed.

main block

Method block with top level of instructions for the run.

method base

Defines the unit for the breakpoints in the method block. The method base is one of the following

- **time** in minutes
- **volume** in liters or milliliters, governed by the selected strategy
- **column volume** set by the user

method block

Series of method instructions. Method blocks can be nested.

Click on the plus sign next to a method block to expand it and view the set of method instructions in the method block.

method instruction

Request for a specific operation in the system.

method template

Basic method which can be used as starting points for customized methods. UNICORN installations for ÄKTA oligopilot plus contain method templates for most synthesis techniques.

method variable

Instruction parameter defined as a variable. The variable is expressed as ***(value)#type {unit}***.

Method variables are powerful when creating a method that contains default parameter values. The default values can be changed to create variants of the same method or adjust the parameter values at the start of a run.

Method variables ease the work of adapting methods to particular oligonucleotide synthesis runs.

Appendix F
Method variables

Base variables

Variable name	Description	Example value
Column_Volume	The volume of the column used	6.3
Column__Volume		6.3
Weight_of_Support	The weight of the support	0.7
Loading_of_Support	The loading of the support	350
Column_Diameter	The diameter of the column	20
Column_Number		
UV_Detritylation	Wavelength	313
CV_Column_Wash	Number of column volumes to use for a column wash	8.00
Detrit_Flow		300
Efficiency_threshold	Threshold value for the efficiency in percent, calculated from the tritely color integration	50

Variables in the ACN washing step after detritylation

Variable name	Description	Example volume
CV_Detrit_Wash	Number of column volumes to use for the ACN washing	6.00

Variables in the coupling step

Variable name	Description	Example value
EQ_Amidite_DNA	Equivalents of amidite. Interval: 1 to 10	1.80
Conc_Amidite_DNA	Amidite concentration. Interval: 0.05 to 0.5 M	0.15
Recycle_Time_DNA	Recycle time in minutes	3.00
CV_Coupling_Wash		4.00

Variables in the oxidation step

Variable name	Description	Example value
CT_Oxidation_DNA		1.00
EQ_Oxidation_DNA	Equivalents of 50 mM I_2. Interval: 1 to 10	2.50
CV_CT_Ox_DNA	Number of column volumes of contact time flowrate	2.00

Variables in the capping step

Variable name	Description	Example value
CT_Capping_DNA		0.50
CV_Capping_DNA		0.50
CV_CT_Capping_DNA		2.00
CV_Capping_Wash		3.00

Items determining what to include in the startup procedure

The following items are displayed in the method editor under ***Start protocol***.

- ***Notes***
- ***Prerun Questions***
- ***Method Info***

- ***Method variables***

These items are used to check and modify what is included in the start up procedure of a method run.

Prompts

The following questions are answered before the synthesis is started. The information entered is used for documentation purposes and does not affect the synthesis.

- No 1: Enter Loading and Lot number of the Support
- No 2: Enter the Weight of the Support
- No 3: Enter the type of support
- No 4: Instrument Identification
- No 5: Enter Column size
- No 6: Amidite A Lot#
- No 7: Amidite C Lot#
- No 8: Amidite G Lot#
- No 9: Amidite G Lot#
- No10: Activator Lot#
- No11: Capping A Lot#
- No12: Capping B Lot#
- No13: Oxidation Lot#
- No14: Thiolation Lot#
- No15: Acetonitrile Lot#
- No16: Detritylation Lot#

Appendix G
Method blocks in ÄKTA oligopilot plus

About this chapter

The method blocks below complete one synthesis cycle.

Depending on the type of chemistry chosen in the sequence editor for the different bases, different method blocks will appear in the text editor below the names of these blocks are listed and their function. The instructions with which these blocks are made up are however not listed since they are equivalent to those used in the example above.

Method block	Description
----- Block : Main ----- 0.00 Base CV, 6.30 {ml} 0.00 Message "Fill your column with DNA-T support", Screen 0.00 Message "Press CONTINUE when ready", Screen 0.00 Pause -1.0 0.00 Call Normal, START_parameters 0.00 Call Normal, Purge_T_U 0.00 Call Normal, Purge_C 0.00 Call Normal, Purge_A 0.00 Call Normal, Purge_G 0.00 Call Normal, Purge_Tetrazole 0.00 Call Normal, purge_solvents_ox 0.00 Call Normal, Column_wash 0.00 Call Normal, Add__DNA_T 0.00 Call Normal, Add__DNA_C 0.00 Call Normal, Add__DNA_C 0.00 Call Normal, Add__DNA_A 0.00 Call Normal, Add__DNA_T 0.00 Call Normal, Add__DNA_G 0.00 Call Normal, Add__DNA_G 0.00 Call Normal, Add__DNA_T 0.00 Call Normal, Add__DNA_A 0.00 Call Normal, Add__DNA_C 0.00 Call Normal, Final_detritylation	The main block contains the highest level of instructions. It is, as well as other blocks in the method, created as a result of the sequence that has been chosen in the sequence editor and the definition of the letters in the sequence via the cross reference list. In the main block, the column volume is defined. In this case the column volume is ***6.30*** mL. The two message instructions will appear on the screen and instructs the operator to fill the column with the correct support and connect it to the system. The rest of the main block is built up of blocks that contain the following: • **Purge of amidites**: This sequence contains A, C, G and T so individual purge methods are called upon for the different monomers that are to be used in the synthesis. The order of blocks is determined by the order they appear in the sequence from the 3′ end (except the very first base, that is attached on the support). • **Purge of activator/tetrazole** • **Purge of solvents used in cycle for oxidized oligos:** This purges all the reagents used for synthesis of oxidized oligos (i.e., oxidation, capping and ACN). • **Column wash:** This is the ACN wash of the column prior to start of synthesis, to remove air-bubbles and moisture from the column. • **DNA synthesis cycles:** As a result of the sequence, methods for the individual bases will called upon in order of appearance starting from the 3´-end.

Method block	Description
	• **Final detritylation:** This method appears if final detritylation has been chosen in the sequence editor. If final detritylation is not chosen, another method, ***Column_Wash***, will appear instead. ***Note:*** *The purge blocks and final detritylation are optional from the sequence editor.*
----- Block : START_parameters ----- 0.00 Base Time 0.00 Block Column_Number 0.00 Block UV_Detrit 0.00 CV (6.3)#Column__Volume {ml} 0.00 Scale (1.70)#Weight_of_Support {g}, (90) #Loading_of_Support {umol/g} 0.00 Coldiameter (20)#Column_Diameter {mm} 0.00 End_block	This block contains the variables that together will define the synthesis scale, namely the weight and the loading of the support. ***Base Time*** as the programming means that the programming base of this block is time. ***Column_Volume***, CV, is in the method again, this time to give the volume to UNICORN to be able to calculate volumes and flowrates besed on the column volume. ***Scale*** : the instruction defines the scale of the synthesis by multiplying the two variables, ***Weight_of_Support*** and ***Loading_of_Support*** for weight of the support with the loading. In ÄKTA oligopilot plus 10 these instructions are already combined so the user is entering the scale directly. ***Coldiameter***, Column_Diameter, is a variable, which defines the column diameter. This is used later on in the method to calculate the linear flow rate used during (e.g., detritylation). In this case with the 6.30 mL column the diameter is 20 mm.
----- Block : Column_Number ----- 0.00 Base Time 0.00 Column (Column_1)#Column_Number 0.05 End_block	A block used to set the column used. It can be Column 1, 2, 3..., 7 or bypass. The variable is, ***Column_Number.*** ***Note:*** *ÄKTA chromatography systems have column 1 connected to position 1, and not 2.*

Method block	Description
----- Block : UV_Detrit ----- 0.00 Base Time 0.00 UV_Wavelength (313)#UV_Detritylation {nm}, 290 {nm}, 0 {nm} 0.10 End_block	This block sets the UV wavelength with the variable ***UV_Detritylation*** used in the method run. Primarily 350 nm is used for larger scale (>150 mmol) and > 350 nm is used when smaller scales are synthesized, which require higher sensitivity. For the smallest scales, use 498 nm for maximum sensitivity.
----- Block : Purge_Detrit_pumpA ----- 0.00 Base Time 0.00 Column Column_Bypass 0.00 Solvent_A Detrit_3.7 0.00 Waste Waste_Detrit 0.10 Flow_A 30.00 {ml/min} 1.00 UV_AutoZero 1.20 Flow_A 0.00 {ml/min} 1.30 Solvent_A ACN_Amidites_3.1 1.40 Flow_A 50.00 {ml/min} 2.00 Flow_A 0.00 {ml/min} 2.10 End_block	This block purges the detritylation solution bypass the column but through the UV monitor and has an autozero instruction included for the UV monitor after 30 mL (1 min x 30mL/min). This step is followed by a 30 mL (0.6min x 50mL/min) acetonitrile wash to clean out pump A and the tubing going in and out of the pump.
----- Block : Purge_T ----- 0.00 Base Time 0.00 Column Column_Bypass 0.00 Amidite T 0.05 Flow_A 5.00 {ml/min} 0.05 Block Amidite_Purge_volume 0.05 Block Amid_ACN_wash_A_T 0.10 End_block	This block primes the T line from valve 1 with X mL at a flow rate of 5.0 mL/min. The priming solutions are directed to the waste outlet, bypassing the column. The flowpath is the acetonitrile position next to the T-amidite. ***Base Time*** means that the programming base of this block is time (min).
----- Block : Amidite_Purge_volume ----- 0.00 Base Volume (1.00)#Amidite_Purge_volume Flow_A 0.00 {ml/min} 1.00 End_block	This block sets the purge volume of the amidites. Default is 1.0 mL but this will be displayed as a variable ***Amidite_Purge_volume.*** ***Base Volume*** means that the programming base of this block is volume (mL).

Method block	Description
----- Block : Purge_A ----- 0.00 Base Time 0.00 Column Column_Bypass 0.00 Amidite A 0.05 Flow_A 5.00 {ml/min} 0.05 Block Amidite_Purge_volume 0.05 Block Amid_ACN_wash_A_T 0.10 End_block	This block primes the A line from valve 1 with X mL at a flow rate of 5.0 mL/min. The priming solutions are directed to the waste outlet, bypassing the column. The flowpath is the acetonitrile position next to the A-amidite. ***Base Volume*** means that the programming base of this block is volume (mL).
----- Block : Amidite_Purge_volume ----- 0.00 Base Volume (1.00)#Amidite_Purge_volume Flow_A 0.00 {ml/min} 1.00 End_block	This block sets the purge volume of the amidites. Default is 1 .0 mL but this will be displayed as a variable. ***Base Volume*** means that the programming base of this block is volume (mL).
----- Block : Purge_C ----- 0.00 Base Time 0.00 Column Column_Bypass 0.00 Amidite C 0.05 Flow_A 5.00 {ml/min} 0.05 Block Amidite_Purge_volume 0.05 Block Amid_ACN_wash_C_G 0.10 End_block	This block primes the C line from valve 1 with X mL at a flow rate of 5.0 mL/min. The priming solutions are directed to the waste outlet, bypassing the column. The flowpath is the acetonitrile position next to the CT-amidite. ***Base Time*** means that the programming base of this block is time (min).
----- Block : Amidite_Purge_volume ----- 0.00 Base Volume (1.00)#Amidite_Purge_volume Flow_A 0.00 {ml/min} 1.00 End_block	This block sets the purge volume of the amidites. Default is 1 .0 mL but this will be displayed as a variable. ***Base Volume*** means that the programming base of this block is volume (mL).

Method block	Description
----- Block : Purge_G ----- 0.00 Base Time 0.00 Column Column_Bypass 0.00 Amidite G 0.05 Flow_A 5.00 {ml/min} 0.05 Block Amidite_Purge_volume 0.05 Block Amid_ACN_wash_C_G 0.10 End_block	This block primes the G line from valve 1 with X mL at a flow rate of 5.0 mL/min. The priming solutions are directed to the waste outlet, bypassing the column. The flowpath is the acetonitrile position next to the G-amidite. ***Base Time*** means that the programming base of this block is time (min).
----- Block : Amidite_Purge_volume ----- 0.00 Base Volume (1.00)#Amidite_Purge_volume Flow_A 0.00 {ml/min} 1.00 End_block	This block sets the purge volume of the amidites. Default is 1.0 mL but this will be displayed as a variable. ***Base Volume*** means that the programming base of this block is volume (mL).
----- Block : Purge_Activator ----- 0.00 Base Time 0.00 Column Column_Bypass 0.00 Solvent_B Activator 0.10 Flow_AB 0.00 {ml/min}, 10.00 {ml/min} 0.10 Block Solvent_Purge_volume 0.20 Solvent_A ACN_Amidites_3.1 0.20 Solvent_B ACN_Reag_4.1 0.30 Flow_AB 20.00 {ml/min}, 20.00 {ml/min} 1.00 Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 1.00 End_block	This block primes the activator line from valve 4 with X mL at a flow rate of 10.0 mL/min. This is followed by 7 mL of ACN from valve 4 pos. 1 as well as 7 mL of ACN from valve 3 pos. 1 at a flowrate of 20 mL/min each. ***Note:*** *This block is always added when purge of amidite is chosen. The acetonitrile washes away the purged amidites from the lines and pumps.* ***Base Time*** means that the programming base of this block is time (min).
----- Block : Solvent_Purge_volume ----- 0.00 Base Volume (5.00)#Solvent_Purge_volume Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 5.00 End_block	This block sets the purge volume of the solvents (e.g., activator, oxidation, capping). Default is 5.0 mL but this will be displayed as a variable (***Solvent_purge_volume***). ***Base Volume*** means that the programming base of this block is volume (mL).

Method block	Description
----- Block : Purge_solvents_Ox ----- 0.00 Base Time 0.00 Block Purge_Oxidation 0.05 Block Purge_CappingAB 0.05 End_block	This block contains the purge method which is relevant prior to synthesis of regular phosphodiester oligonucleotides (i.e., capping, oxidation and ACN). will appear which will purge the Thiolation instead of the oxidation lines. • For synthesis of regular phosphodiester oligonucleotides, the method ***Purge_Oxidation*** is invoked, purging the oxidation lines. • If thiolated DNA is to be synthesized , the purge method ***Purge_solvents_thio*** is invoked, purging the thiolation lines. A third type, called ***Purge_solvents_mix***, is included if the sequence contains both phosphodiester and thiolated bases.
----- Block : Purge_Oxidation ----- 0.00 Base Time 0.00 Column Column_Bypass 0.00 Solvent_B Ox 0.10 Flow_AB 0.00 {ml/min}, 10.00 {ml/min} 0.10 Block Solvent_Purge_volume 0.20 Solvent_A ACN_Amidites_3.1 0.20 Solvent_B ACN_4.4 0.30 Flow_AB 20.00 {ml/min}, 20.00 {ml/min} 1.00 Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 1.00 End_block	This block primes the oxidation line from valve 4 with X ml at a flow rate of 10.0 mL/min. This is followed by 7 mL (20mL/min * 0.7min / 2pumps = 7mL) of ACN from valve 4 pos. 4 as well as 7 mL of ACN from valve 3 pos. 1, in order not to mix different reagents in the valve that can cause precipitation.
----- Block : Solvent_Purge_volume ----- 0.00 Base Volume (5.00)#Solvent_Purge_volume Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 5.00 End_block	This block sets the purge volume of the solvents (e.g. tetrazole, oxidation, capping). Default is 5.0 mL but this will be displayed as the variable ***Solvent_purge_volume***. ***Base Volume*** uses volume based programming for this block.

Method block	Description
----- Block : Purge_CappingAB ----- 0.00 Base Time 0.00 Column Column_Bypass 0.00 Solvent_A Cap_A 0.10 Flow_AB 10.00 {ml/min}, 0 {ml/min} 0.10 Block Solvent_Purge_volume 0.20 Solvent_B Cap_B 0.30 Flow_AB 0.00 {ml/min},10 {ml/min} 0.30 Block Solvent_Purge_volume 0.40 Solvent_A ACN_Amidites_3.1 0.40 Solvent_B ACN_4.4 0.50 Flow_AB 20.00{ml/min}, 20.00 ml/min} 1.20 Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 1.20 End_block	This block primes the capping A and B lines from valve 3 pos. 2 and valve 4 pos. 2 with X mL each, at a flow rate of 10.0 mL/min. This is followed by 7 mL of ACN from valve 4 pos. 1 as well as 7 mL of ACN from valve 3 pos. 1.
----- Block : Solvent_Purge_volume ----- 0.00 Base Volume (5.00)#Solvent_Purge_volume Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 5.00 End_block	This block sets the purge volume of the solvents (e.g., activator, oxidation, capping). Default is 5.0 mL but this will be displayed as the variable ***Solvent_purge_volume***. ***Base Volume*** uses volume based programming for this block.

Method block	Description
----- Block : Column_wash ----- 0.00 Base SameAsMain 0.00 Block Column_Number 0.00 Waste Waste_ACN 0.00 Solvent_A ACN_Amidites_3.1 0.00 Solvent_B ACN_Reag_4.1 0.00 PFlow_AB 10.00 {Bar} (8.00)#CV_Column_Wash Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 8.00 End_block	***Base SameAsMain*** sets the programming base to that of the main method, in this case column volumes. The first line calls ***Column_Number***, selecting the the column to wash with acetonitrile. The flowpath is set to take acetonitrile from valve 3 pos. 1 and valve 4 pos. 1. ***PFlow_AB*** starts a maximum flowrate creating no more than 10 Bar of backpressure on pumps A and B . The flow stops after ***8.00*** column volumes, which is a variable. ***CV_Column_Wash*** is a variable for the number of column volumes of the column wash. Larger colums require fewer column volumes for a wash compared to smaller columns.

Method block	Description
----- Block : Add__DNA_T ----- 0.00 Base Time 0.00 Set_mark "" T "" 0.10 Block Detritylation 0.20 Block Detrit_wash 0.20 Base_Id _T 0.25 Block Coupling_Recycle_DNA_T 0.30 Block Oxidation_DNA 0.35 Block Capping_DNA	This block contains all the method blocks needed to complete one synthesis cycle for the addition of one DNA T monomer. ***Base Time*** uses time based programming for this block. The following blocks or instructions in the method are executed in order of appearance: • ***Detritylation*** • ***Detrit wash*** • ***Coupling_recycle_DNA_T*** • ***Oxidation_DNA*** • ***Capping_DNA*** These methods are identical for all bases of the same type (e.g. ,phosphodiester DNA except for the identity of the monomer in the coupling block). ***Base_Id _T*** gives information to the software about the kind of base. This information is used when calculating the last efficiency values. Only bases with the same Id are compared when the last coupling efficiency is calculated. ***Set_mark***: the text inside the quotation marks is included in the chromatogram at the given breakpoint (min or mL).

Method block	Description
----- Block : Detritylation ----- 0.00 Base Time 0.00 Set_mark "Detritylation" 0.10 Block Detrit_peak_start_UV 0.10 Block Detrit_peak_end_UV 0.10 Integration_OFF 0.30 Flow_A 0.00 {ml/min} 0.35 End_block	This block contains method blocks which identify the start and end of the detritylation peak. ***Set_mark***: the text inside the quotation marks is included in the chromatogram at the given breakpoint (min or mL). The on-line integration of the trityl peak is also set to ***OFF*** towards the end of the block. After ***Integration_OFF*** there is an extra 0.3 minutes of detritylation to make sure that there is a complete detritylation of the growing oligo-chain in the column. ***Base Time*** uses time based programming for this block.

Method block	Description
----- Block : Detrit_peak_start_UV ----- 0.00 Base SameAsMain 0.00 ******Cycle_Start****** 0.00 Waste Waste_Detrit 0.00 Solvent_A Detrit_3.7 0.00 LFlow_A (400)#Detrit_Flow {cm/h} 1.20 Watch_UV1 Greater_than, 250 {mAU}, END_BLOCK 12.00 Watch_off UV1 12.00 Solvent_A ACN_Amidites_3.1 12.00 Solvent_B ACN_Reag_4.1 12.00 PFlow_A 10.00 {Bar} 16.90 Flow_A 40.00 {ml/min} 17.00 Message "No_peak_detected", Screen 17.00 Pause -1 {Minutes} 17.00 Solvent_A Detrit_3.7 17.00 LFlow_A 400 {cm/h} 17.00 End_block	This block contains instructions for valve and flow rate settings for detritylation as well as instructions for starting integration of the detritylation peak. • ***Base SameAsMain*** sets the programming base to that of the main method, in this case column volumes (CV). • *********Cycle_Start********* is an internally used instruction which identifies the start of a new synthesis cycle from where the retention is calculated. • ***Waste Waste_Detrit*** sets the waste valve in the detrit position, valve 8 pos. 2, to allow separation of chlorinated waste from non-chlorinated). • ***Solvent_A Detrit 3.7*** identifies the correct valve settings for detritylation. • ***LFlow_Det 400 {cm/h}*** identifies the pump and flow rate for detritylation. For detritylation, a linear flow rate is chosen (***LFlow***) and set as a variable. This allows the same basic method to be used, independent of column size. • ***Integration_Start*** sets the starting point from where the method should start looking for a peak that fulfills the conditions set as ***Start_Level*** in System Settings. • ***Watch_UV1 Greater than 250 {mAU), END_BLOCK*** means that the block should end when the detritylation peak has reached a absorbance on UV1 higher than 250. • ***Watch_off UV1*** turns off the watch function, in this case after 12 column volumes.

Method block	Description
	If the watch conditions do not occur (no detritylation peak), the system switches to a 5 column volume ACN wash and then Pause (the pause is infinite) the run and a message appears on the screen with the text ***No peak detected***. If the synthesis shall proceeed, instructions follow for turning valves for more detritylation with a linear flowrate.
----- Block : Detrit_peak_end_UV ----- 0.00 Base SameAsMain 0.00 Integration_ON 2.00 Watch_UV1 Less_than, 250 {mAU}, END_BLOCK 8.00 Watch_off UV1 8.00 End_block	• When the watch conditions in the peak start block have occurred, that block is ended and the block ***Detrit_Peak_end*** is started. • ***Base SameAsMain*** sets the programming base to that of the main method, in this case column volumes. • ***Watch_UV1 Less than 250{mAU), END_BLOCK*** means that the block ends when the conductivity has dropped below 250. • ***Watch_off UV1*** turns off the watch function, in this case after 8 column volumes.

Method block	Description
----- Block : Detrit_wash ----- 0.00 Base SameAsMain 0.00 Set_mark "Det_Wash" 0.00 Solvent_A ACN_Amidites_3.1 0.00 Solvent_B ACN_Reag_4.1 0.00 Flow_AB 20 {ml/min}, 20 {ml/min} 1.00 PFlow_A 10.00 {Bar} 2.50 Watch_Efficiency Less_than, (50)#Efficiency_threshold {%}, PAUSE 2.80 Watch_off Efficiency (6.00)#CV_Detrit_Wash Flow_A 0.00 {ml/min} 6.00 Waste Waste_ACN 6.00 End_block	This block contains instructions for ACN wash of the column after detritylation. • ***Set_mark*** places the quoted text in the chromatogram at the given time or volume breakpoint. • ***Base SameAsMain*** sets the programming base to that of the main method, in this case column volumes. • ***Solvent_A ACN Amidites_3.1*** sets valve 3 to pos. 1, which is the valve setting for ACN to pump A. • ***Solvent_A ACN Reag_4.1*** sets valve 4 to pos. 1, which is the valve setting for ACN to pump B. • ***Flow_AB 20ml/min, 20 ml/min*** sets the flowrates of pump A and pump B to 20 mL/min each. • ***PFlow_A*** sets a maximum flowrate, creating no more then 10 bar of backpressure on pump A. The Flow stops after 6.00 CV which is a variable. • ***Watch_Efficiency Less_than 50%*** and the variable ***Efficiency_threshold*** checks if the efficiency, calculated from the tritely color integration, is at least as the chosen value. If not, the synthesis will be paused. The instruction is placed 2.8 column volumes into the wash block to guarantee that the synthesis is paused with acetonitrile in the column, not acid detrit solution. • ***Watch_off Efficiency*** turn the efficiency watch off. • ***Waste Waste_ACN*** sets valve 8 to position 1 to collect in the ACN waste. • ***#CV_detrit_wash*** is the variable that defines the number of column volumes for the ACN wash., 6,00 CV for the 1.2 and 6.3 mL column or more for smaller columns and less for larger.

Method block	Description
----- Block : Coupling_Recycle_DNA_T ----- 0.00 Base Time 0.00 Set_mark "Coupling" 0.00 Block DNA_parameters 0.00 Block Amid_ACN_wash_T_U 0.00 Block Column_Number 0.05 Block Amidite_amount_T_U 0.10 Block Recycle_DNA 0.20 Block Coupling_wash 0.25 End_block	This block contains sub blocks required for • addition of amidite • addition of tetrazole • recirculation of the coupling reagents over the column • the ACN required between and the next step in the cycle. The ***Set_mark*** instruction places the quoted text in the chromatogram at the given time or volume breakpoint.

Method block	Description
----- Block : DNA_parameters ----- 0.00 Base Time 0.00 Eq_Amidite (1.50)#Eq_Amidite_DNA {Eq} 0.00 %_Activator (60)#Percent_Activator_DNA {%} 0.00 Amidite_Conc (0.1)#Conc_Amidite_DNA {M} 0.00 End_block	This block contains variables that define the number of amidite equivalents, the amount of activator amount and the concentration of amidite. • ***Base Time*** sets time as the programming base. • ***Eq_Amidite*** contains the variable ***#Eq_Amidite_DNA***, which determines the number of amidite equivalents to use during coupling. • - 1.5 amidite equivalents are used if the column volume is equal to or larger than 6.3 mL. - 3 amidite equivalents are used if the column volume is 1.2 mL. - 10 amidite equivalents are used for the small cassette. • ***%Activator*** contains the variable ***#Percent_Activator_DNA {%}*** which determines the amount of activator, in percent, of the coupling volume that shall be mixed with the amidite for the coupling reaction. In this case the amount is 60%, which is valid for all standard amidites. Some activators and types of amidites may require adjustments. • ***AmiditeConc*** contains the variable ***#Conc_Amidite_DNA {M}*** which takes into account the concentration of the amidite solution. - ÄKTA oligopilot plus 10 typically uses 100 mM. - ÄKTA oligopilot plus 100 typically uses 0.1 to 0.15 M when using the 6.3 mL column. When using the FineLINE 35 oligo column, 0.2 M should be used.

Method block	Description
	The combination of variables in this block and the start parameters is the base upon which the computer calculates the correct amount of amidite and activator used for each coupling reaction.
----- Block : Amid_ACN_wash_T ----- 0.00 Base Time 0.00 Column Column_Bypass 0.00 Amidite ACN_T/U 0.10 Flow_A 24.00 {ml/min} 0.60 Flow_A 0.00 {ml/min} 0.60 End_block	This block contains instruction for ACN wash from the relevant amidite valve position before adding the coupling reagents. The wash goes directly to waste, and reduces the risk of cross contamination when the amidite valve changes position. • ***Base Time*** sets time as the programming base. • ***Column_Bypass*** sets the flowpath column bypass, which is reagent directly to waste. • ***Amidite ACN_T/U***, sets flow path to the ACN position connected closest to the T/U amidite. • ***Flow_A*** sets the flow rate of the reagent pump, in this case to 24.00 mL/min. After (0.6 – 0.1) x 24= 12 mL, the pump flow is switched to 0.00 mL/min.
----- Block : Amidite_amount_T ----- 0.00 Base Time 0.00 Amidite T/U 0.00 Block Coupling_Reag_Vol 0.00 Amidite ACN_T/U 0.05 Block Coupling_push 0.10 End_block	This block contains blocks and instructions to add coupling reagents and push them into the column with acetonitrile. • ***Base Time*** sets time as the programming base. • ***Amidite T*** sets the correct flow path for the T/U amidite. • ***Amidite ACN_T*** sets the correct flow path for the ***ACN_T/U***.

Method block	Description
----- Block : Coupling_Reag_Vol ----- 0.00 Base Time 0.10 Coupling Time_0.5 {min} 0.30 End_block	***Coupling*** instructs the computer to calculate the correct volumes of amidite and activator to add, based on the synthesis scale and the DNA parameters. This instruction needs to be separated to a unique breakpoint, i.e. a unique point in time (0.00 , 0.10 , 0.30). The flow rate used results in a contact time of 0.50 min the first time the coupling reagents pass through the column. The contact time is calculated on the remaining coupling volume when 1 equivalent is consumed. ***Note:*** *For maximum efficiency, the total coupling should be around 1 CV.*
----- Block : Coupling_push ----- 0.00 Base Volume 0.00 FlowThrough 0.50 {min} 2.00 Solvent_B ACN_Reag_4.1 12.00 LFlow_AB 0 {cm/h} 12.00 End_block	This block pushes the amidite and tetrazole into both column and coupling loop at a flowrate that will create a minimum of 0.5 min contact time for the coupling reagents when 1 eq is consumed. • The block starts using acetonitrile in pump A and activator in pump B. • After 2 mL (1 mL from each pump), valve 4 switches to add acetonitrile also to pump B. The total push volume is 12 mL and a typical flow rate using PrimerSupport 30, HL in the 6.3 mL column is 2.3 + 2.3 mL/min. This gives a time for this step of just over 2½min.

Method block	Description
----- Block : Recycle_DNA ----- 0.00 Base Time 0.00 Set_mark "Recycle" 0.00 Recycle On, 250 {cm/h} (3.00)#Recycle_Time_DNA Recycle Off, 0 {cm/h} 3.00 End_block	This block defines the conditions, for recirculating the coupling reagents using pump B. • ***Base Time*** sets time as the programming base. • The ***Set_mark*** instruction places the quoted text in the chromatogram at the given time or volume breakpoint. • ***Recycle On , 250{cm/h}*** closes the recirculation loop and starts a linear flow rate of 250 cm/h. • ***Recycle On*** sets the flow path for recycle. • ***Recycle_time*** is a variable that defines the time for recycling. • ***Recycle Off, 0 {cm/h}***, OFF stops the flow of the reagent pump and sets the flow path for flow through column.
----- Block : Coupling_wash ----- 0.00 Base SameAsMain 0.00 Set_mark "Coupling_wash" 0.00 PFlow_AB 10.00 {Bar} (4.00)#CV_Coupling_Wash Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 4.00 End_block	This block contains blocks for ACN wash of the reagent pump directly to waste, followed by ACN wash of the column with pressure regulated flow. The number of column volumes is represented as the variable ***#CV_Coupling_Wash***. • ***Base SameAsMain*** sets the programming base to that of the main method, in this case column volumes. • The ***Set_mark*** instruction places the quoted text in the chromatogram at the given time or volume breakpoint. • ***PFlow_AB*** starts a maximum flowrate creating no more then 10 bar of backpressure on pumps A and B. The flow stops after 4.00 column volumes.

Method block	Description
----- Block : Oxidation_DNA ----- 0.00 Base Time 0.00 Set_mark "OX" 0.00 Block Amount_Ox_DNA 0.10 Block Ox_push 0.15 Block Contacttime_Ox_DNA 0.20 Block Ox_wash 0.25 Flow_B 0.00 {ml/min} 0.30 End_block	This block contains blocks adding the oxidation solution, setting the contact time and initiating a subsequent washing step. • ***Base Time*** sets time as the programming base. • The ***Set_mark*** instruction places the quoted text in the chromatogram at the given time or volume breakpoint.
----- Block : Amount_Ox_DNA ----- 0.00 Base Time 0.10 Oxidation (1.00)#CT_Oxidation_DNA {min}, (2.00)#Eq_Oxidation_DNA {Eq} 0.30 End_block	This block adds the required amount of oxidation solution. • ***Base Time*** sets time as the programming base. • ***CT_Oxidation*** and its variable ***#CT_Oxidation_DNA*** sets the contact time for the reagent in the column. • ***Eq_Oxidation*** is an instruction set as a variable, which calculates the correct amount of oxidation solution based on the number of equivalents. This calculation is based on the earlier variables for the synthesis scale. The calculation is also based on the use of a 50 mM solution of Iodine (I2). Synthesis scale (mmol) * Eq_oxidation / 50mM = mL oxidation. • ***Oxidation*** translates the calculations from ***Eq_Oxidation*** into flow rate instructions for the addition. The flow rate at this step is the same as for the defined oxidation contact time. This instruction also sets the correct flow path for oxidation to column (valve 4.5). ***Note:*** *For the ÄKTA oligopilot plus 10 methods, oxidation reagent is used on a volume base. This decreases the time needed for the step and secures the chemical reaction when the column and reagent volumes are very small.*

Method block	Description
----- Block : Ox_push ----- 0.00 Base Volume 0.00 Set_mark "CT_OX" 4.00 End_block	The block ***Ox_push*** is a block programmed with volume base. The block is used to push the oxidation in to the column. The flowrate has been set in the previous **Amount_Ox_DNA** block by the ***Oxidation*** instruction calculated from the parameters.
----- Block : Contacttime_Ox_DNA ----- 0.00 Base SameAsMain (2.00) #CV_CT_Ox_DNA End_block	This block governs the flow rate for the oxidation reaction as the solution is pushed through the column with ACN. This flow rate is also used for the addition which is done by the instruction Oxidation. • ***Base SameAsMain*** sets the programming base to that of the main method, in this case column volumes. • ***CV_ct_Ox_DNA*** is a variable that determines the number of column volumes for which the contact time flow rate of the oxidation shall be carried out.
----- Block : Ox_wash ----- 0.00 Base SameAsMain 0.00 Set_mark "OX_wash" 0.00 PFlow_AB 10.00 {Bar} 1.00 PFlow_B 10.00 {Bar} 1.10 Watch_Cond Less_than, 50 {uS/cm}, END_BLOCK 2.00 Watch_off Cond 2.00 End_block	This block completes the wash of the column with ACN after the oxidation reaction. • ***Base SameAsMain*** sets the programming base to that of the main method, in this case column volumes. • ***PFlow_AB*** starts a maximum flowrate creating no more then 10 bar of backpressure on pumps A and B. The flow changes after 1.00 CV. • ***PFlow_B*** continues at a maximum flowrate creating no more then 10 bar of backpressure on pump B. The flow stops after 2.00 CV or when the conductivity is less then 50 mS/cm. • ***Watch_Cond Less than, 50.00 {µS/cm}, END_BLOCK*** is a conductivity watch function. The wash continues until the conductivity has dropped below ***50.00 µS/cm***. If the conductivity for some reason will not decrease below the set level, the wash will continue for 2 column volumes and than stop anyway.

Method block	Description
----- Block : Capping_DNA ----- 0.00 Base Time 0.00 Set_mark "CAP" 0.00 Block Amount_Cap_DNA 0.05 Block Cap_push 0.10 Block Contacttime_Cap_DNA 0.15 Block Capping_wash 0.25 End_block	This block adds the capping soltion, sets the contact time and performs the subsequent washing. • ***Base Time*** sets time as the programming base. • The ***Set_mark*** instruction places the quoted text in the chromatogram at the given time or volume breakpoint.
----- Block : Amount_Cap_DNA ----- 0.00 Base Time 0.10 Capping (0.50)#CT_Capping_DNA {min}, (0.50) #CV_Capping_DNA {CV} 0.20 End_block	This block adds the required amount of capping solution. • ***CT_Capping*** and the variable ***#CT_Capping_DNA*** sets the contact time for the reagent in the column. • ***#CV_Capping*** is an instruction, set as a variable, that calculates the correct amount of capping solution based on the number of column volumes. This calculation is in in turn based on previous variables. • ***Capping*** translates the calculations from ***CV_Capping*** and ***CT_Capping*** into flow rate instructions for the actual addition. The flow rate at this step is the same as for the capping contact time defined below. This instruction also sets the correct flow path for the capping to column.
----- Block : Cap_push ----- 0.00 Base Volume 0.00 Set_mark "CT_Cap" 4.00 End_block	This block pushes the oxidation into the column, using the flowrate set in the previous step ***Amount_Cap_DNA***.
----- Block : Contact-time_Cap_DNA ----- 0.00 Base SameAsMain (2.00) #CV_CT_Capping_DNA End_block	The variable ***#CV_CT_Capping*** in this block is used to set the number of column volumes of ACN to use when pushing the capping reagents through the column.

Method block	Description
----- Block : Capping_wash ----- 0.00 Base SameAsMain 0.00 Set_mark "Cap_wash" 0.00 PFlow_AB 10.00 {Bar} (3.00) #CV_Capping_Wash Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 3.00 End_block 0.00 Base SameAsMain 0.00 Set_mark "Cap_wash" 0.00 PFlow_AB 10.00 {Bar} (3.00) #CV_Capping_Wash Flow_AB 0.00 {ml/min}, 0.00 {ml/min} 3.00 End_block	This is the last block in the synthesis cycle, performing a pressure regulated column wash with acetonitrile. ***Base SameAsMain*** sets the programming base to that of the main method, in this case column volumes. ***PFlow_AB*** starts a maximum flowrate creating no more than 10 bar of backpressure on pumps A and B . The flow stops after 4.00 CV, set via the variable ***#CV_capping_wash***.

Description of special blocks

Method block	Description
----- Block: Final_detritylation: -----	This block is the last in the method if final detritylation was chosen in the sequence editor. It contains the regular detritylation block as well as the block for the subsequent ACN wash (detrit wash).
----- Block :Column_wash -----	This block is the last in the method if final detritylation was NOT chosen, and contains instruction for an ACN wash only.
----- Block :Thiolation_DNA -----	This block appears instead of ***Oxidation_DNA*** for those bases that have been chosen to be thiolated in the sequence editor. It contains the same functions as the oxidation block. The volume of thiolation reagent is programmed in column volumes rather than equivalents.
----- Block :Purge_Solvents_Ox_Thio -----	This block is used to purge the reagent lines when a combination of oxidized and thiolated bases are used.
----- Block : Add__RNA_Base (a, c, g, u etc.) -----	This block is equivalent to the corresponding DNA block, except for those coupling and oxidation blocks specific for RNA.

Method block	Description
----- Block: Coupling_recycle_RNA_ (a, c, g, u etc.) ----	This block is used to set up the coupling conditions for regular RNA amidites. It is equivalent to the corresponding DNA block with regard to the types of instructions and variables used.
----- Block: RNA_parameters -----	This block contains variables that define the number of amidite equivalents, tetrazole amount, and amidite concentration for coupling of regular RNA amidites. It is equivalent to the corresponding DNA block with regard to the types of variables used.
----- Block: Thiolation_2oMe -----	This block is used for oxidation of regular RNA bases. It is equivalent to the corresponding DNA block with regard to the types of instructions and variables used.
----- Block: Thiolation_RNA -----	This block is used for thiolation of regular RNA bases. It is equivalent to the corresponding DNA block with regard to the types of instructions and variables used.
----- Block: Add__2oMe_(a, c, g, u etc.) -----	This block is equivalent to the corresponding DNA block except for the coupling and oxidation blocks that are specific for 2´-OMe-RNA amidites.
----- Block: Coupling_recycle_2oMe_ (a, c, g, u etc.) -----	This block is used to set up the coupling conditions for 2´-OMe-RNA amidites. It is equivalent to the corresponding DNA block with regard to the types of instructions and variables used.
----- Block: 2oMe_parameters -----	This block contains variables that define the number of amidite equivalents, tetrazole amount, and amidite concentration for coupling of 2´-OMe-RNA amidites. It is equivalent to the corresponding DNA block with regard to the types of variables used.
----- Block: Oxidation_2oMe -----	This block is used for oxidation of 2´-OMe-RNA bases. It is equivalent to the corresponding DNA block with regard to the types of instructions and variables used.

Method block	Description
----- Block : Thiolation_2oMe -----	This block is used for thiolation of 2´-OMe RNA bases. It is equivalent to the corresponding DNA block with regard to the types of instructions and variables used.

Appendix H
Strategy calculations

About this chapter

This chapter contains calculations and formulas used in synthesis, together with example calculations.

Note: *To see more strategy help, highlight the instruction in UNICORN and press F1.*

In this chapter

H.1 Scale

Example parameters

Parameter	Value	Allowed range
Column volume	6.3 mL	-
Solid support weight	700 mg	0.1 to 150 g
Loading	350 µmol/g	1 to 500 µmol/g

Equation

Scale = Solid Support Weight x Loading

Scale = 0.7 x 350 = 245 µmol

Note: *The strategy for ÄKTA oligopilot plus 10 in UNICORN has the* ***Scale*** *instruction instead of weight and loading.*

H.2 Coupling

Overview

The calculated flowrates are calculated on the volume of coupling reagents when 1 eq of the amidites is consumed. If 1.5 eq is used, and the total coupling reagent volume is 1500 mL, then one eq is 1000 mL. The remainder is 500 mL. This means the flowrates are calculated as follows:

- 500 mL divided by the CT, in this example 1 minute = 500 mL/min.
- 500 mL/min divided 40/60 between the amidite and activator pump equals 200 and 300 mL/min, respectively.

Input data

Parameter	Unit	Name
Synthesis scale	µM	$scale$
Amidite concentration	M	C_{amid}
Amidite equivalents	-	eq_{amid}
Activator concentration	%	C_{act}

Reagent volume calculation

$$V_{coupl}\,[ml] = \frac{scale \times eq_{amid}}{C_{amid} \times 1000} + \left(\frac{C_{act}}{100 - C_{act}} \times \frac{scale \times eq_{amid}}{C_{amid} \times 1000} \right)$$

Amidite flow calculation (flow A)

$$F_{amid}\,[ml/min] = \left(V \times \frac{eq_{amid} - 1}{eq_{amid}} \times \frac{100 - C_{act}}{100}\right) / CT_{coupl}$$

Note: *CT_{coupl} is 0.25, 0.50 or 1.00 minutes*

Activator flow calculation (flow B)

$$F_{act}\,[ml/min] = F_{amid} \times \frac{C_{act}}{100 - C_{act}}$$

Example calculation

Input data

Parameter	Value
Scale	260
Amidite equivalents	1.5
Amidite concentration	0.15 M = 150 µmol/mL
Activator concentration	60%

Amidite volume

$V_{amidite}$ = scale × $EQ_{amidite}$ = (amidite amount) / (amidite concentration)

= 1.5 × 260 = 390/150 = **2.6 mL of amidite**

Activator volume

$V_{activator}$ = scale × $V_{amidite}$ = 1.5 × 1.5 mL = **3.9 mL of activator**

Total volume

V_{total} = $V_{amidite}$ + $V_{activator}$ = 2.6 + 3.9 = **6.5 mL**

Flow rate base volume

This volume will be used to calculate the flow rate during adition of amidite and activator.

Base volume = V_{total} - ($V_{activator}$ + $V_{amidite}$)/scale

= 6.5 - (3.9 + 2.6)/1.5 = **2.17 mL**

Flow A and flow B

The instruction Coupling time is set to 0.25, 0.5 or 1 min and is used to calculate the flow rate as follows.

Activator concentration = 60% = 0.60

Flow A = (0.40 × 2.17 mL) / 0.25 min = **3.42 mL/min**

Flow B = (0.60 × 2.17 mL) / 0.25 min = **5.21 mL/min**

H.3 Flowthrough

Equation

$$F\ [mL] = V_{coupl} \times \left(eq_{amid} - \frac{1}{eq_{amid}} \right) \Big/ T_{flowthrough}$$

$T_{flowthrough}$: 0.5 to 10 min

Default = 1 min

H.4 Recycle flow

Equation

$$F[ml/min] = F_{lin} \times \frac{r^2\pi}{60}$$

F_{lin} : 0 to 1000 cm/h

r: column radius in cm

H.5 Oxidation reagent

Input data

Parameter	Unit	Name
Synthesis scale	µM	$scale$
Oxidation reagent concentration	M	C_{ox}
Oxidation reagent equivalents	No unit Range: 1 to 5	eq_{ox}
Contact time	Range: 0.1 to 5	CT

Flow rate calculation

$$F_{ox}\,[ml/min] = \frac{eq_{ox} \times scale}{T \times 0.05}$$

eq_{ox}: 1 to 5

Volume calculation

$$V_{ox}\,[ml] = \frac{eq_{ox} \times scale}{0.05}$$

Contact time calculation

$$CT[min] = \frac{V_{ox}}{F_{ox}}$$

CT: 0.1 to 5 minutes

Example calculation

Input data

Parameter	Value
Oxidation reagent concentration	50 µmol/mL

Parameter	Value
Oxidation reagent equivalents	2
Synthesis scale	260 µmol (0.7 g × 357 µmol/g)
Contact time	1 min

Volume

$V_{ox} = eq_{ox} \times scale / C_{ox} = 2 \times 260 / 50 =$ **10.4 mL**

Flow rate

$F_{ox} = V_{ox} / CT = 10.4 / 1 =$ **10.4 mL/min**

H.6 Capping reagent

Input data

Parameter	Unit	Variable name
No. of column volumes	No unit Range: 0.1 to 2	n_{CV}
Contact time	Range: 0.1 to 2	CT

Flow rate calculation

$$F_{thio}\,[ml/min] = \frac{V_{thio} \times n_{CV}}{CT}$$

Capping reagent volume

The capping reagent volume is equal to the number of column volumes.

Contact time calculation

$$CT\,[min] = \frac{n_{CV}}{F_{cap}}$$

CT: 0.1 to 2 minutes

n: 0.1 to 2

Example calculation

Capping reagents A and B are added as a CV multiple at a programmed contact time.

Input data

Parameter	Value
No. of column volumes	0.5
Contact time	0.5 min

Capping reagent volume

$V_{cap} \times CV = V_{capA} + V_{capB} = 0.5 \times 6.3$ mL = 3.15 mL

$V_{capA} = V_{capB} = 3.15 / 2 =$ **1.58 mL**

Flow rate, pump A

$flow_{capA} = V_{capA} / time_{contact} = 1.58 / 0.5 =$ **3.16 mL/min**

Flow rate, pump B

$F_{capB} = V_{capB} / CT = 1.58 / 0.5 =$ **3.16 mL/min**

H.7 Thiolation reagent

Input data

Parameter	Unit	Variable name
Thiolation reagent volume	mL	V_{thio}
Number of column volumes	No unit Range: 0.1 to 10	n_{CV}
Contact time	min Range: 0.5 to 10	CT

Flow rate calculation

$$F_{cap}\,[ml/min] = \frac{2\,(V \times\ n_{CV})}{CT}$$

Example calculation

Input data

Parameter	Value
Thiolation reagent volume	6.3 mL
Number of column volumes	1
Contact time	3 min

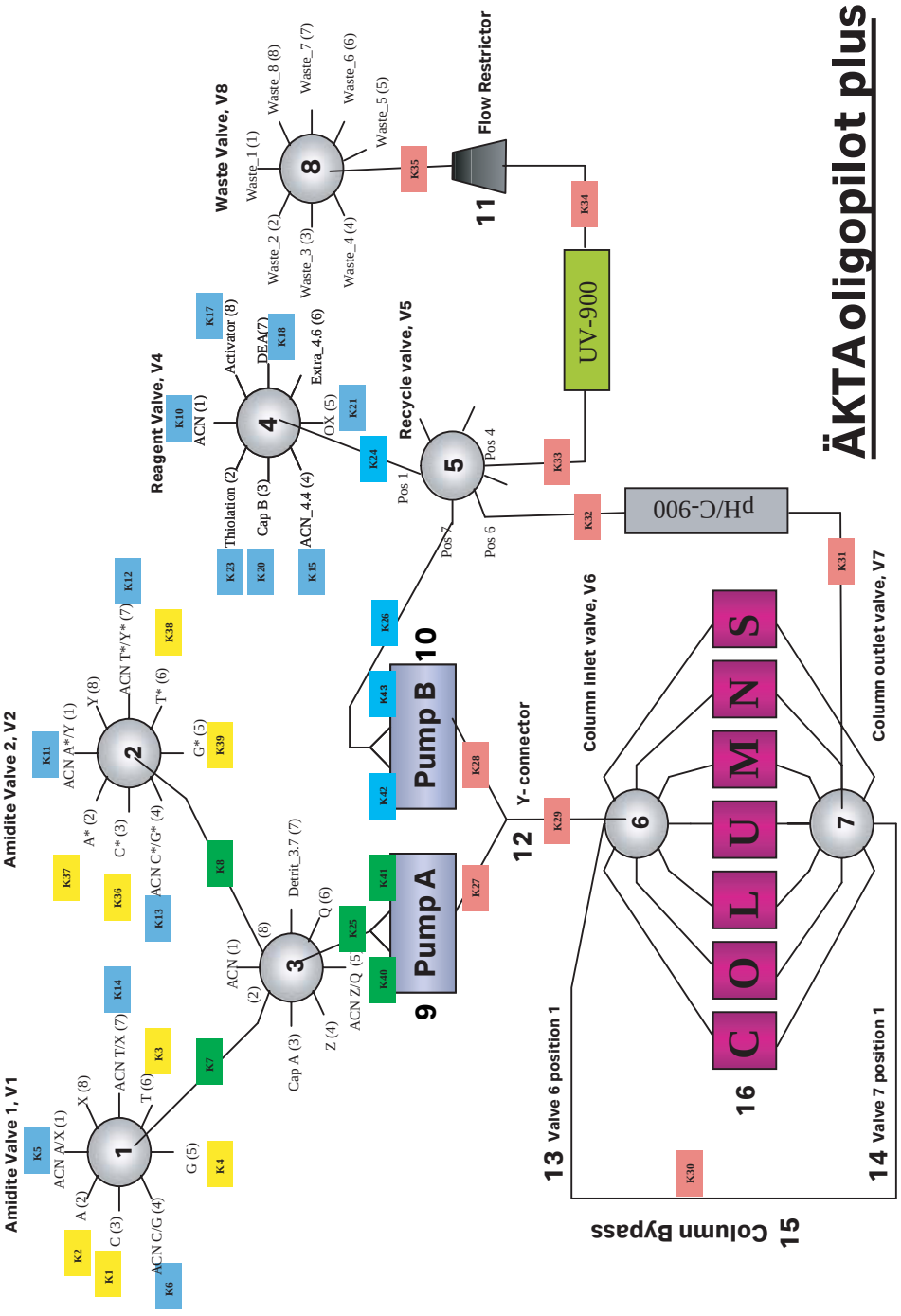
Flow rate, pump B

Flow rate = V_{thio} / $T_{contact}$ = 6.3 / 3 = **2.1 mL/min**

Appendix I
Connection diagrams

In this chapter

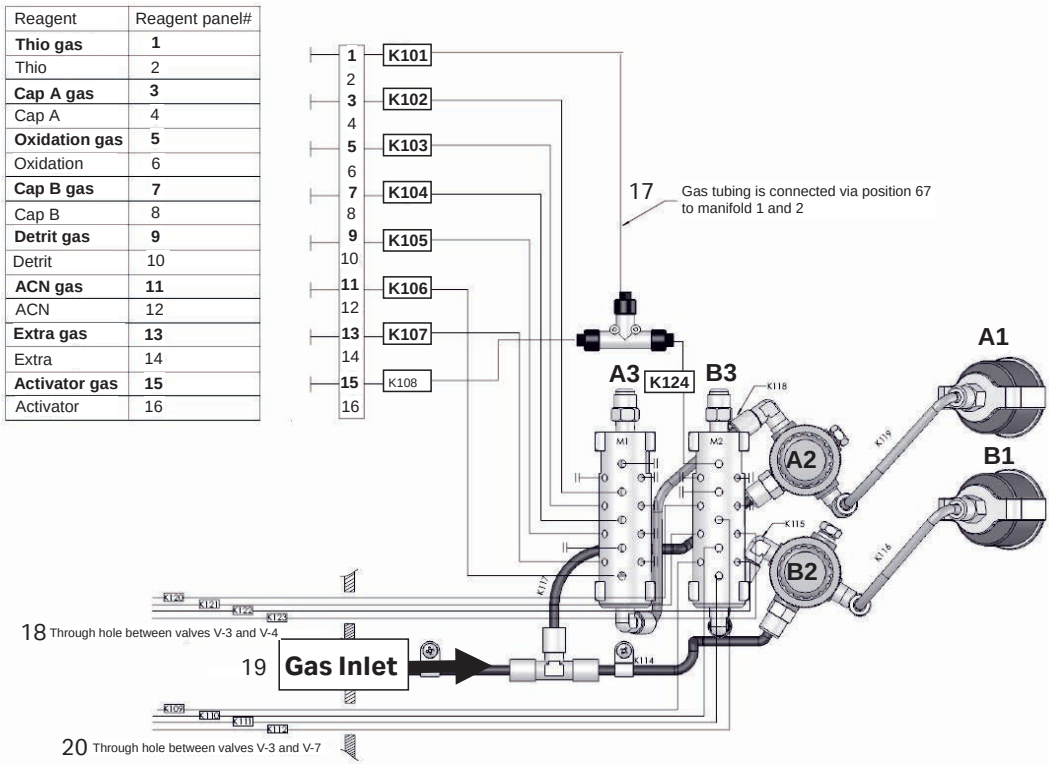
I.1 Liquid flow path and components



Part	Description
1	Amidite valve 1, V1
2	Amidite valve 2, V2
3	Valve 3, V3
4	Reagent valve, V4
5	Recycle valve, V5
6	Column inlet valve, V6
7	Column outlet valve, V7
8	Waste valve, V8
9	Pump A
10	Pump B
11	Flow restrictor
12	Y-connector
13	Valve 6, position 1
14	Valve 7, position 1
15	Column bypass
16	Columns

I.2 Gas connection diagram

Reagent	Reagent panel#
Thio gas	**1**
Thio	2
Cap A gas	**3**
Cap A	4
Oxidation gas	**5**
Oxidation	6
Cap B gas	**7**
Cap B	8
Detrit gas	**9**
Detrit	10
ACN gas	**11**
ACN	12
Extra gas	**13**
Extra	14
Activator gas	**15**
Activator	16



Part	Description
1	**Thio gas**
2	Thio
3	**Cap A gas**
4	Cap A
5	**Oxidation gas**
6	Oxidation
7	**Cap B gas**
8	Cap B
9	**Detrit gas**
10	Detrit
11	**ACN gas**
12	ACN
13	**Extra gas**
14	Extra
15	**Activator gas**
16	Activator
17	Gas tubing, connected via position 67 to manifold 1 and 2
18	Through holes between valves V-3 and V-4
19	Inert gas inlet
20	Through holes between valves V-3 and V-7
A1	**Manifold 1** pressure gauge
B1	**Manifold 2** pressure gauge
A2	Manifold 1 gas regulator
B2	Manifold 2 gas regulator
A3	Manifold 1
B3	Manifold 2

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28959748 AF V:8 02/2021