ÄKTA oligopilot plus

OLIGONUCLEOTIDE SYNTHESIS

ÄKTA™ oligopilot™ plus is a flexible, fully automated oligonucleotide synthesizer designed for use in research and process development laboratories and in oligonucleotide production facilities (Fig 1). ÄKTA oligopilot plus is available in two configurations: ÄKTA oligopilot plus 10 for synthesis in the 1 to 50 µmol range; and ÄKTA oligopilot plus 100 for synthesis in the 50 µmol to 9 mmol range.

ÄKTA oligopilot plus allows the automated synthesis of up to seven different oligonucleotides in sequence using as many as 12 different synthesis monomers with high coupling efficiencies and low reagent consumption (Fig 2).

ÄKTA oligopilot plus oligonucleotide synthesizers offer:

- Flexibility in synthesis methods, chemistry, and scale
  - phosphodiester and/or phosphorothioate DNA, RNA oligonucleotides
- Good synthesis economy
  - low amidite consumption
  - generates significantly less waste
  - re-circulation of monomer solutions
- High throughput
  - cycle time for a 1 µmol synthesis is under 5 min
- Simple method scouting and scale-up
  - synthesis of up to seven oligonucleotides sequentially
  - simple method scale-up without additional method optimization
  - UNICORN™ software transparent to scale
- On-line monitoring for process analytical technology (PAT)
  - conductivity, UV-vis, and pressure

Fig 1. ÄKTA oligopilot plus provides fully automated DNA/RNA oligonucleotide synthesis in research and process development.

Operational overview

ÄKTA oligopilot plus synthesizers are compact, pump-driven systems that employ flow-through reactor technology (described later). This results in significantly reduced reagent consumption — an important consideration when performing oligonucleotide synthesis at large scale. Several column reactors and disposable Oligosynt™ columns and cassettes (Fig 2) are available for synthesis over a wide range of scales (see Ordering information). The adjustable FineLINE™ 35 oligo column has been specifically developed to allow oligo synthesis at scales from 250 µmol to 3.8 mmol using ÄKTA oligopilot plus. During synthesis, the coupling mixture is added to the synthesis support as a tight "reagent zone" that is pushed through the column. In this way the coupling mixture is not diluted as the reaction proceeds.

Flexibility in synthesis methods, chemistry and scale

ÄKTA oligopilot plus is supplied with optimized, preprogrammed standard method templates for phosphodiester (Fig 3 to 6) and/or phosphorothioate DNA (Fig 7), RNA oligonucleotides

(Fig 8) for several synthesis scales and columns. These standard methods can be easily modified to suit your needs. Alternatively, you can easily design and program your own synthesis methods.

The availability of 12 monomer ports simplifies synthesis of chimeric and labeled oligonucleotides. The seven-port valve in ÄKTA oligopilot plus also allows recirculation of high value monomer solutions through the synthesis reactor, thereby maximizing utilization of the coupling mixture. This is an important economy consideration when using high-value monomers in the synthesis reaction.

Fig 2. ÄKTA oligopilot plus synthesizer with different types of multiple columns and additional monomers.

ÄKTA oligopilot plus is available in two scale versions permitting synthesis at the 1 to 50 µmol (ÄKTA oligopilot plus 10) and 50 µmol to 9 mmol scales using Primer Support 200 at loadings of 200 µmol/g (ÄKTA oligopilot plus 100). Kits are available to enable conversion of ÄKTA oligopilot plus 10 to ÄKTA oligopilot plus 100.

Good large-scale synthesis economy with low amidite consumption and high coupling efficiency

The flow-through technology of ÄKTA oligopilot plus offers amidite savings compared with a batch reactor synthesizer. DNA phosphoramidite consumption can be reduced to as little as 1.5 molar equivalents of phosphoramidite per coupling while still maintaining a coupling efficiency of > 99%. For RNA synthesis, coupling efficiency is > 98% using only 3 molar equivalents.

Because ÄKTA oligopilot plus is so efficient, it generates significantly less waste than batch reactor synthesizers.

Method scouting and process development tool

The use of motorized multichannel valves and flexible UNICORN software provides the option to automate synthesis of up to seven oligonucleotides sequentially on ÄKTA oligopilot plus. The use of UNICORN and flow-through reactor technology in combination with Primer Support allows for simple linear method scale-up without the need for additional method optimization.

On-line monitoring and PAT

On-line conductivity and UV-monitors enable Process Analytical Technology (PAT) and provide an accurate, continuous display of the coupling efficiency in each synthesis step. In the event of a poor coupling reaction, signals from the on-line monitors can be used to pause the synthesis before additional reagents are consumed.

The multi-wavelength UV-visible monitor operates at three wavelengths simultaneously (254 to 700 nm) and the wavelengths selected can be changed during a run. This can be used to confirm sequence accuracy, monitor reaction cycles and wash steps, and to confirm identity of reagents being added at each cycle.

High throughput and short cycle times

Flow-through operation offers speed as well as efficiency. The cycle time for a 1 µmol synthesis using ÄKTA oligopilot plus 10 is under 5 min. The cycle time for a 2.5 mmol synthesis of a 20-mer is less than 25 min using ÄKTA oligopilot plus 100.

Small-scale synthesis

The new advanced ÄKTA oligopilot plus 10 system, with optimized design and method templates, gives excellent results at 1-µmol scale with low reagent consumption (Fig 3, Table 1).

Highly reproducible linear scale up with maintained efficiency

Method scale-up from, for example from 250 µmol (using 6.3-mL column) to 6 mmol (150 mL, OPII-adjustable column) and further to 30 mmol (Oligopilot 400, 785-mL FineLINE 100 adjustable column) is simple with ÄKTA oligopilot plus. UNICORN synthesis methods can be scaled simply by increasing flow rates and reagent volumes (Figs 4 to 6).

Waste handling and reuse of monomer solutions

The eight port waste valve in ÄKTA oligopilot plus allows separation of chlorinated from nonchlorinated hydrocarbons, thus reducing waste disposal costs. Since it is possible to separate waste with the remaining six outlets, excess monomer may be collected for reuse, if required.

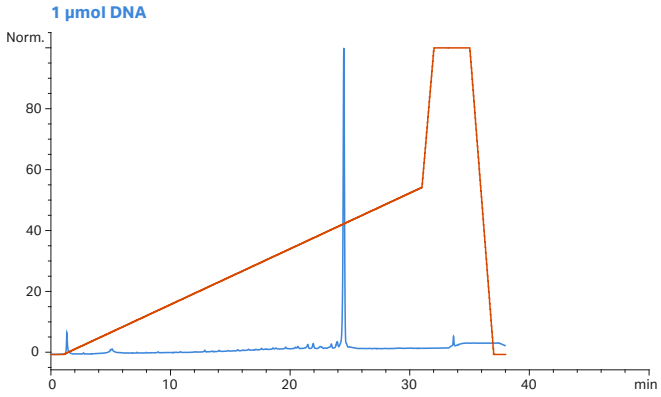


Fig 3. Analytical ion exchange chromatography of crude phosphodiester 20-mer synthesized using ÄKTA oligopilot plus 10 at the 1 µmol synthesis scale. Full Length Product (FLP) =86%, n-1=2.8%, yield=155 AU_{260}/µmol. TEST 20 sequence 3′ TT TGA AGC GAA TTA GCC ATA 5′. For analytical conditions, see the footnote.

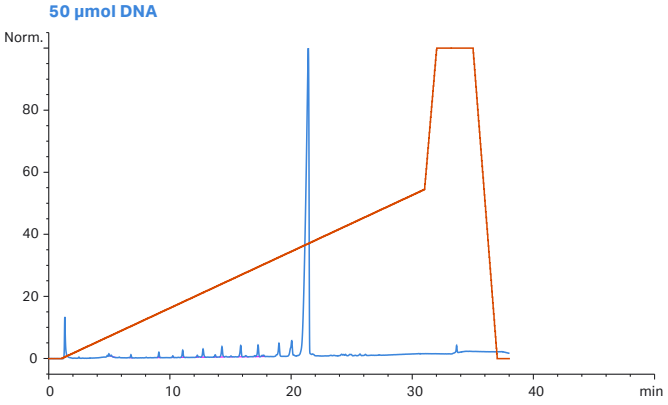


Fig 4. Analytical ion exchange chromatography of crude phosphodiester 13-mer synthesized using ÄKTA oligopilot plus 10 at the 50 µmol synthesis scale. FLP=89%, n-1=2.6%, yield 186 AU_{260}/µmol. TEST 13 sequence: 3′ T TTT AAA CCC GGG 5′. For analytical conditions, see the footnote.

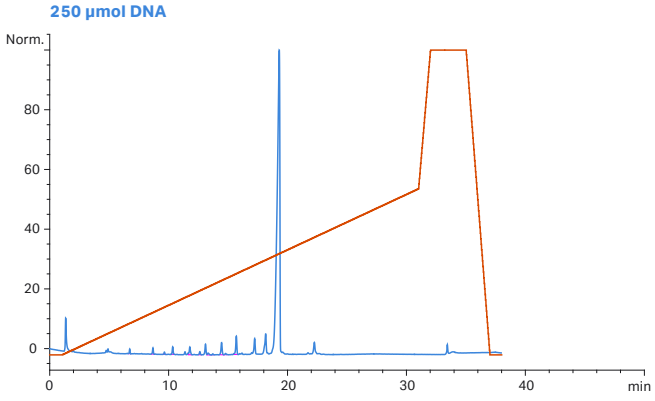


Fig 5. Analytical ion exchange chromatography of crude phosphodiester 13-mer synthesized using ÄKTA oligopilot plus 100 at the 250 µmol synthesis scale. FLP=85%, n-1=3.2%, yield 85 AU_{260}/µmol. TEST 13 sequence: 3′ T TTT AAA CCC GGG 5′. For analytical conditions, see the footnote.

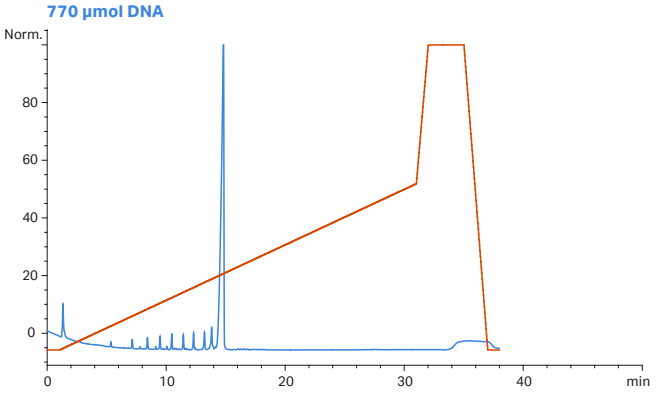


Fig 6. Analytical ion exchange chromatography of crude phosphodiester 13-mer synthesized using ÄKTA oligopilot plus 100 at the 770 µmol synthesis scale. FLP=87%, n-1=3.2%, yield 99 AU_{260}/µmol. TEST 13 sequence: 3′ T TTT AAA CCC GGG 5′. For analytical conditions, see the footnote.

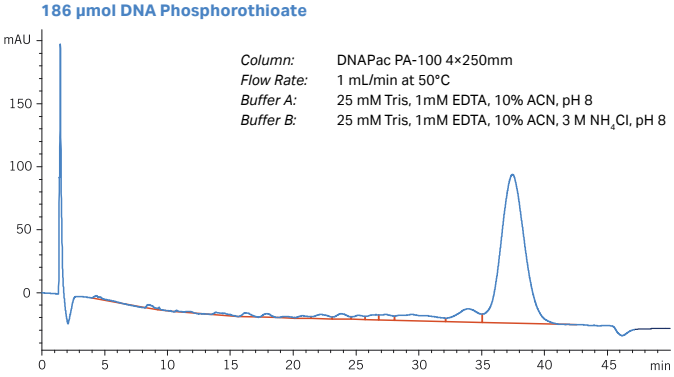


Fig 7. Analytical ion exchange chromatography of crude phosphorothioate 20-mer synthesized using ÄKTA oligopilot plus 100 at the 186 µmol synthesis scale. PO=0.33%, yield=142 AU_{260}/µmol. TEST 20 sequence 3′ TT TGA AGC GAA TTA GCC ATA 5′.

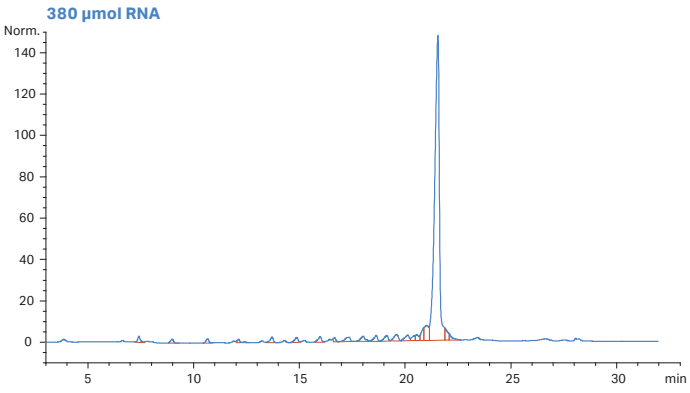


Fig 8. Analytical ion exchange chromatography of crude 21-mer siRNA synthesized using ÄKTA oligopilot plus 100 at the 380 µmol synthesis scale. Full length product (FLP)=76%, n-1=3.7%, yield 125 AU_{260}/ µmol. siRNA sequence: 3′ TGU GGC UUG AGU UUC UUC CGG 5′. For analytical conditions, see the footnote.

Figures 3-6, 8 analytical conditions:

Column: DNAPac PA-100 4×250mm
Flow rate: 1 mL/min at 50°C
Buffer A: 10 mM N_aClO_4, 1 mM Tris
Buffer B: 300 mM N_aClO_4, 1 mM Tris

Flow-through reactors — the key to good synthesis economy and scalability

Low amidite consumption

The unique flow-through reactor design permits almost quantitative couplings using as little as 1.5 molar equivalents of phosphoramidite monomer at large scales. The superior efficiency of a flow-through reactor over a batch reactor is due to the behavior of the coupling mixture in the reactor. Figure 9 compares the performance of a flow-through reactor to that of a batch reactor.

In the flow-through reactor of ÄKTA oligopilot plus (Fig 9A), coupling mixture is added to the solid support as a reagent zone (shown in blue). The coupling mixture is pushed through the column as a well-defined zone. Reagents at the front of this zone are consumed by the coupling reaction at a continuous rate. As this zone moves down the reactor, new reagents continuously replace those used. Even liquid distribution ensures that coupling efficiences remain the same throughout the reactor. This continuous replacement of consumed reagents by new reagents keeps the concentration constant throughout the zone. This in turn maintains a constant reaction rate during the whole reaction.

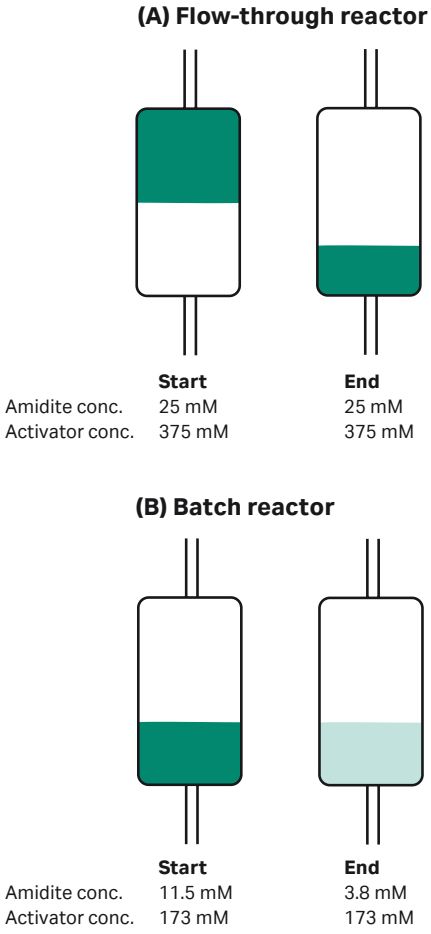
In batch reactors on the other hand, consumed reagents are not replenished; amidite concentration decreases continuously, which reduces the overall reaction rate (Fig 9B).

High flow rates, high washing efficiency, and low ancillary reagent consumption

Because ÄKTA oligopilot plus pumps may be operated at high flow rates (2 × 10 mL/min in ÄKTA oligopilot plus 10 and 2 × 100 mL/min in ÄKTA oligopilot plus 100), efficient washing and short cycle times are possible. Furthermore, high flow rates during detritylation ensure that the contact time between synthesis support and acidic detritylation solution is minimized, thus avoiding depurination.

The economical advantages of ÄKTA oligopilot plus technology are emphasized as the scale of oligonucleotide synthesis increases. At large synthesis scales such as 2.5 mmol, ÄKTA oligopilot plus consumes as little as 2 molar equivalents of oxidation solution per cycle. This low consumption is a result of the flow-through technology where acetonitrile is used to push reagents through the column as a moving reagent zone at a controlled flow rate. This also results in very efficient washing.

In contrast, commercially available synthesizers employing molar batch-reactor technology require immersion of the entire support bed in reagent, which results in much higher reagent consumption. In addition, washing times and volumes of acetonitrile consumed are reduced by as much as three-fold in ÄKTA oligopilot plus. The volumes of waste solvents generated are also reduced significantly.



(A) Flow-through reactor. The coupling mixture moves down as a reagent zone, continuously replacing reagents consumed by the reaction. The amidite concentration is the same at the end of the reaction as at the beginning.

(B) Batch reactor. Reagents consumed by the coupling reaction are not replaced. Amidite concentrations fall to low levels.

Fig 9. Comparing the efficiency of a flow through reactor with that of a batch reactor. At the end of the reaction, when one molar equivalent of amidite has been consumed, the active concentration of amidite is more than six-fold higher in the flow-through compared with the batch reactor.

Table 1. Typical reagent consumption and cycle time for a 1-µmol synthesis of oligonucleotide

Reagent	Consumption (per cycle)
Amidite 0.1 M	5 mg
Activator	0.3 mL
Oxidation	0.2 mL
Cap A	0.4 mL
Cap B	0.4 mL
Detritylation	3 mL
Acetonitrile	11 mL
Cycle time	~4.5 min

User-friendly, validatable software

UNICORN software is used to design, control, monitor, and evaluate synthesis procedures using ÄKTA oligopilot plus.

UNICORN is also used with OligoPilot 400 and OligoProcess™ and all purification systems from Cytiva.

The software employs a sequence editor interface that enables you to define an oligonucleotide sequence up to 250 bases long. The sequence entered is used to create a ready-to-run method automatically from the preprogrammed UNICORN method templates:

- Method templates provide method frameworks for most common applications and eliminate the need to start method programming from scratch
- Modular method definition in the method templates with separate modules for detritylation, coupling, oxidation/thiolation, capping, and wash/purge steps
- User-defined alarms and warning limits for monitoring the synthesis process
- On-line display of coupling efficiency
- Batch operation and process documentation in accordance with the requirements of Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP). Full compliance to 21 CFR Part 11.

In addition, UNICORN offers a comprehensive security system:

- Electronic signature and record system
- Password control for all users, with access authorization for different users' method and results files
- Customized definition of access control levels
- Traceable audit trail

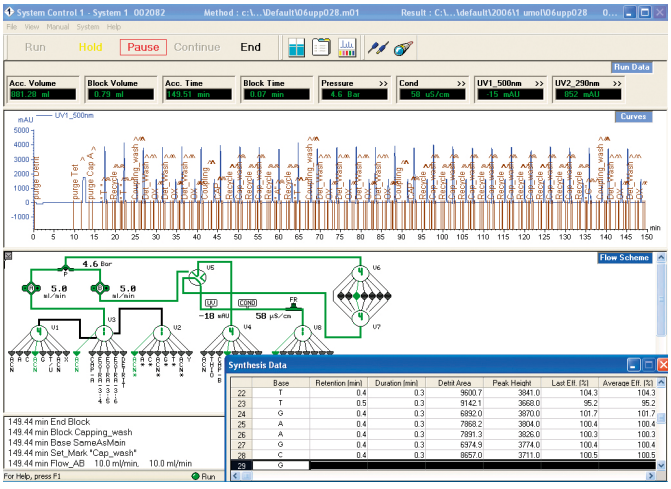


Fig 10. The UNICORN System Control window.

FineLINE 35 oligo

FineLINE 35 oligo is an adjustable oligosynthesis column for use with ÄKTA oligopilot plus (Fig 11). Synthesis may be performed with bed volumes from 10 mL to 100 mL for synthesis at scales in the range from 250 µmol to 3.8 mmol, making scale-up simple.

Fig 11. FineLINE 35 oligo column is used with ÄKTA oligopilot plus for efficient oligonucleotide synthesis and easy scale-up.

Primer Support

We offer a range of standard and custom synthesis supports based on uniform polystyrene beads. High coupling efficiencies and high product purity are achieved with these solid supports. Cytiva also offers a custom–derivatization service where Primer Support 200 can be loaded with almost any molecule to different degrees of substitution according to your specific requirements.

Validation support

Cytiva has assisted many diagnostic and pharmaceutical manufacturers in validating their production processes. This regulatory support expertise is also available to users of ÄKTA oligopilot plus. Installation and Operational Qualification support services and documentation are available. In addition, Regulatory Support Files are available for Primer Support. Please contact your local Cytiva office for additional information.

Cytiva — the total supplier

ÄKTA oligopilot plus, OligoPilot 400 and OligoProcess are part of a product range that extends from synthesis reagents and instrumentation to chromatography media and ÄKTAdesign purification systems for purification of the oligonucleotide product at low scales and up to full production.

Additional information regarding oligonucleotide purification is available in our Application Notes. Please contact your local Cytiva office for more information.

Technical specifications

Chemistry

Chemistry:	Synthesis of DNA, RNA, phosphodiester, and phosphorothioat oligonucleotides using β-cyanoethyl, phosphoramidites
Synthesis scales:	1 µmol to 9 mmol depending on degree of substitution of the solid support used
Coupling efficiency:	> 99% for DNA > 98% for RNA
Cycle time:	4.5 to 25 min for standard DNA
Reagent stability in instrument:	At least 2 wk

Software

Controlled by UNICORN software on an external computer. The software is menu and mouse driven and allows method creation, synthesis system control, monitoring, and evaluation. UNICORN software is supplied on a CD. System-specific Strategy and Template methods are supplied on a separate CD.

Instrument

Dimensions: (W × H × D)	450 × 610 × 480 mm
Weight:	63 kg
No. of column reactors:	Up to seven
Column reactor	See ordering information
No. of monomer ports:	Eight fitted as standard. Option to use additional monomer inlets up to a total of 12
Reagent delivery and washing:	Active pumping with a flow rate range of 0.01 to 100 mL:
ÄKTA oligopilot plus 10:	2 × 10 mL/min
ÄKTA oligopilot plus 100:	2 × 100 mL/min
Monitoring and control system:	Conductivity monitoring. UV-vis monitoring of DMTr. Automatic calculation of coupling efficiency. Pause capability if synthesis fails. Pressure.
Protective system:	Argon or Nitrogen atmosphere
Delivery systems:	Eight low dead-volume motorized valves
Control system:	UNICORN software on an external computer
Waste diversion system:	Separates chlorinated from nonchlorinated hydrocarbons. Also possible to separate six other solvents, e.g., monomer solutions.
Methods:	Optimized, standard methods for synthesis in the range 1 µmol to 9 mmol Customize methods by creating new methods or changing existing standard methods
Warranty:	One-year parts and labor

Ordering information

Product	Code no.
ÄKTA oligopilot plus 100	18-1136-79
ÄKTA oligopilot plus 10	18-1140-42
Conversion kits	
ÄKTA oligopilot plus 10 to 100	28-4100-87
Bottles and holders	
Bottle cap 4 × 5/16" connections 1 × o.d.1/16" (gas), 1 × o.d.1/16", or o.d.1/8": and 2 × o.d.3/16" tubing	18-1137-01
Amidite bottle slide incl. connectors	18-1138-46
Columns	
Column Reactor Assembly small 1 reactor assembly for small cassettes	18-1142-91
Primer Support Packing Kit (column + filter) 10 empty cassettes, 0–0.14 mL	18-1035-19
Column Reactor complete, 1.2 mL Stainless steel	18-1101-10
Column Reactor complete, 6.3 mL Stainless steel	18-1101-13
Column Reactor complete, 12 mL Stainless steel	18-1101-16
Column Reactor complete, 24 mL Stainless steel	18-1101-19
Column Reactor complete, 48 mL Stainless steel	18-1101-22
FineLINE 35 oligo 10–100 mL	18-1137-13
OP II Adjustable Column 30–200 mL	18-1107-60
Column holders	
Column Holder For 1.2, 6.3, 12, 24, and 48 mL stainless steel column reactors	18-1138-45
Column Holder For 5 × Column Reactor Assembly small	18-1113-18

Related product literature

	Code no.
Application note: Strategies for large-scale purification of synthetic oligonucleotides	18-1116-00
Application note: Automated pilot-scale purification of synthetic phosphorothioate oligonucleotides	18-1111-25
Application note: Optimization and scale-up of siRNA synthesis	28-4057-96
Data file: Primer Support 200	18-1167-86
Data file: Custom Primer Support for oligonucleotide synthesis	18-1167-79
Product guide: Oligonucleotide synthesis	18-1172-75
Application note: Small-scale DNA synthesis	28-4094-57
Application note: Small-scale RNA synthesis	28-4094-58

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