



1- μ mol synthesis of DNA using ÄKTA oligopilot plus 10

Intellectual Property Notice: The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

cytiva.com

GE and the GE Monogram are trademarks of General Electric Company. Other trademarks listed as being owned by General Electric Company contained in materials that pre-date the Danaher acquisition and relate to products within Cytiva's portfolio are now trademarks of Global Life Sciences Solutions USA LLC or an affiliate doing business as Cytiva. Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate. All other third-party trademarks are the property of their respective owners.
© 2020 Cytiva
All goods and services are sold subject to the terms and conditions of sale of the supplying company operating within the Cytiva business. A copy of those terms and conditions is available on request. Contact your local Cytiva representative for the most current information.
For local office contact information, visit [cytiva.com/contact](https://www.cytiva.com/contact)

1- μ mol synthesis of DNA using ÄKTA oligopilot plus 10

The pilot scale synthesis instruments from GE Healthcare, ÄKTA™ oligopilot™ 10 and ÄKTA oligopilot 100 have been upgraded in regards to hardware, software, and synthesis methods. As a result of the upgrade, ÄKTA oligopilot plus 10 has become a highly efficient system for 1- μ mol scale synthesis of DNA oligonucleotides.

Synthesis

DNA oligonucleotides of various lengths were synthesised using an ÄKTA oligopilot plus 10 systemt. The methods for 1 μ mol scale synthesis of phosphodiester DNA

oligonucleotides have been optimized and, relative to the original version of the ÄKTA oligopilot 10 system, significant improvements have been made in terms of reagent consumption, quality of the oligonucleotides synthesized, and cycle time. The reagents used for synthesis and deprotection are listed in Table 1 below.

Synthesis of oligonucleotides in the 1- μ mol scale was carried out in disposable cassettes. Empty cassettes and appropriate filters are available from GE Healthcare (code number 18-1035-19). Best results have been obtained with cassettes packed with Custom Primer Support™ 40s, 25 mg/cassette. Prior to start of synthesis, the cassettes

Table 1. Reagents used for synthesis, cleavage and deprotection

Reagent	Composition/quality	Supplier	Code number
Solid support, 1g	Custom Primer Support dA 40s,	GE Healthcare	17-5214-37
Solid support, 1g	Custom Primer Support dC 40s,	GE Healthcare	17-5214-38
Solid support, 1g	Custom Primer Support dG 40s,	GE Healthcare	17-5214-39
Solid support, 1g	Custom Primer Support T 40s,	GE Healthcare	17-5214-40
Amidite dA ^{bz} , 1g	0.1M, Standard phosphoroamidite, >98%	Pierce Milwaukee	27-1730-04
Amidite dC ^{bz} , 1g	0.1M, Standard phosphoroamidite, >98%	Pierce Milwaukee	27-1732-04
Amidite dG ^{ibu} , 1g	0.1M, Standard phosphoroamidite, >98%	Pierce Milwaukee	27-1734-04
Amidite T, 1g	0.1M, Standard phosphoroamidite, >98%	Pierce Milwaukee	27-1736-04
Acetonitrile, 2.5L	DNA synthesis grade	EMD Chemicals	AXO152/2505
Detritylation, 1L	3% DCA in toluene	EMD Chemicals	BIO832/1005
Activator, 1L	BTT (benzylthiotetrazole) 0.3 M, in ACN	EMD Chemicals	BIO166/1005
Capping A, 0.5L	20% NMI in ACN	EMD Chemicals	BIO224/0505
Capping B, 2 x 0.2L	20% Ac ₂ O, 30% 2,6-lutidine in ACN	EMD Chemicals	BIO347/0200 BIO349/0200
Oxidation, 1L	50 mM I ₂ in pyridine/water 9:1	EMD Chemicals	BIO424/1005
Deprotection, 0.5L	20% diethylamine in ACN	EMD Chemicals	NC0017-0505
Cleavage & deprotection 2?	Concentrated ammonium hydroxide	EMD Chemicals	AX1196-1



were placed in column holders (code number 18-1142-91) connected to ÄKTA oligopilot plus 10. The reagent consumption for the optimized synthesis cycle for 1- μ mol synthesis is shown in Table 2.

Table 2. Reagent consumption for 1- μ mol synthesis on ÄKTA oligopilot plus 10

Reagent	Amount
Acetonitrile	11 ml
Detritylation	3 ml
Amidite	6.0 eq, 5 mg
Activator	0.3 ml
Oxidation	0.2 ml
Capping A	0.4 ml
Capping B	0.4 ml

The time for completion of one synthesis cycle is 4.5 min. This means that a 20 mer can be synthesized in less than 1.5 h.

Cleavage and base deprotection

After synthesis, the solid support (still in the cassette) was transferred to a microcentrifuge tube with the flange up. The tube was placed in a small table centrifuge and spun for about 1 min at medium speed (2000 rpm) to remove the acetonitrile inside the cassette. The cassette was then transferred to a screw-cap microcentrifuge tube, 1 ml concentrated ammonium hydroxide was added, and the ammonium hydroxide allowed to enter the cassette centrifuging for about 1 min at medium speed. The tube containing the cassette was then heated in an oven at 55°C to 60°C for 16 h (overnight) and then allowed to cool to room temperature. The cassette was then transferred to a new microcentrifuge tube and the cleavage solution still inside the cassette was collected at the bottom of the tube after centrifuging for about 1 min at medium speed. The cassette was removed from the tube, and the cleavage solution was combined with the solution in the original tube.

Yield determination

After cleavage and deprotection, the synthesis yields were determined by measuring the absorbance at 260 nm of an aliquot of the crude mixture diluted in water. In order to make the yields comparable for different synthesis scales, they are expressed as A_{260} units/ μ mol.

HPLC purity analysis

After cleavage and deprotection, the purity of the crude reaction mixtures were analyzed by ion exchange (IEX) HPLC using the conditions shown in Table 3.

Table 3. Conditions used for IEX HPLC analysis

HPLC system	Agilent 1100
Column	DNA Pac™ PA100
Injection volume	2 μ l
Sample concentration	20 to 30 A_{260} units/ml
Buffer A	1 mM Tris, 10 mM NaClO ₄ ,
Buffer B	1 mM Tris, 300 mM NaClO ₄
Flow rate	1000 μ l/min
Gradient	1% to 55% B in 30 min for < 40-mers 1% to 70% B in 40 min for > 40-mers
Column temperature	50°C

Results

With the procedures described above, very high synthesis efficiency is obtained. Average coupling efficiency is in the 99% to 99.5% range. Examples of HPLC analyses of crude material after synthesis and deprotection are shown opposite for a 20-mer (Fig 1) and an 80-mer (Fig 2).

20-mer phosphodiester

Yield: 155 OD/ μ mol
Purity: 86%
N-1: 2.8%

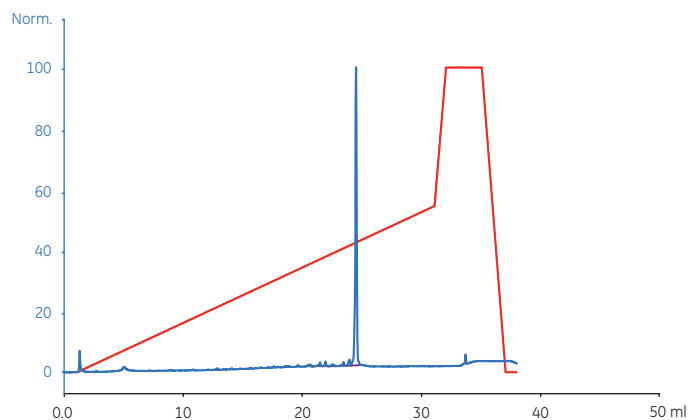


Fig 1. HPLC analysis of 20-mer phosphodiester.

80-mer phosphodiester

Yield: 618 OD/ μ mol
Purity: 55%
N-1: 3.5%

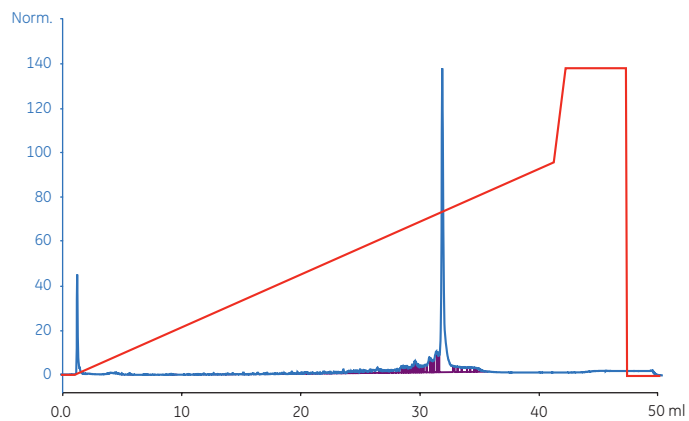


Fig 2. HPLC analysis of 80-mer phosphodiester.

Conclusions

The use of optimized synthesis methods for 1- μ mol scale synthesis of DNA oligonucleotides in ÄKTA oligopilot plus 10 in combination with the use of Custom Primer Support 40s gives oligonucleotides of very high yield and purity.

www.gehealthcare.com/oligo

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

AKTA, OligoPilot, Primer Support are trademarks of GE Healthcare companies. GE, imagination at work, and GE monogram are trademarks of General Electric Company.

DNA Pac is a trademark of Dionex Corporation.

The use of AKTA oligopilot and OligoProcess instruments is licensed under US Patent Nos. 4,458,066 and 4,973,679, and corresponding patents issued in other countries, when synthesis of polynucleotides is performed thereon using solid phase supports provided from a licensed supplier. Patented reagents suitable for use with these instruments are available from licensed sources. No other license is granted to the purchaser either directly or by implication, estoppel or otherwise.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. GE Healthcare reserves the right, subject to any regulatory and contractual approval, if required, to make changes in specifications and features shown herein, or discontinue the product described at any time without notice or obligation. Contact your local GE Healthcare representative for the most current information.

© 2006 General Electric Company – All rights reserved.

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala, Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Ltd
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

Asia Pacific Tel +65 6275 1830 Fax +65 6275 1829 • Australasia Tel +61 2 9899 0999 Fax +61 2 9899 7511 • Austria Tel 01/57606-1619 Fax 01/57606-1627 • Belgium Tel 0800 73 888 Fax 02 416 82 06 • Canada Tel 800 463 5800 Fax 800 567 1008
Central, East, & South East Europe Tel +43 1 972720 Fax +43 1 97272 2750 • Denmark Tel 45 16 2400 Fax 45 16 2424 • Finland & Baltics Tel +358 (0)9 512 39 40 Fax +358 (0)9 512 39 439 • France Tel 01 69 35 67 00 Fax 01 69 41 96 77 • Germany
Tel 089 96281 660 Fax 089 96281 620 • Greater China Tel +852 2100 6300 Fax +852 2100 6338 • Italy Tel 02 27322 1 Fax 02 27302 212 • Japan Tel +81 3 5331 9336 Fax +81 3 5331 9370 • Latin America Tel +55 11 3933 7300 Fax +55 11 3933 7304 • Middle
East & Africa Tel +30 210 9600 687 Fax +30 210 9600 693 • Netherlands Tel 0800 82 82 82 1 Fax 0800 82 82 82 4 • Norway Tel 815 65 555 Fax 815 65 666 • Portugal Tel 21 417 7035 Fax 21 417 3184 • Russia & other C.I.S. & N.I.S Tel +7 (495) 956 5177
Fax +7 (495) 956 5176 • Spain Tel 93 594 49 50 Fax 93 594 49 55 • Sweden Tel 018 612 1900 Fax 018 612 1910 • Switzerland Tel 0848 8028 12 Fax 0848 8028 13 • UK Tel 0800 616928 Fax 0800 616927 • USA Tel 800 526 3593 Fax 877 295 8102

