

GE Healthcare



illustra NAP-10 Columns


Gravity flow columns for the purification of oligonucleotides and small DNA fragments, desalting and buffer exchange

Product booklet

Codes: 17-0854-01 (20 purifications)
17-0854-02 (50 purifications)



Page finder

1. Legal	3
2. Handling	4
2.1. Safety warnings and precautions	4
2.2. Storage conditions	4
2.3. Expiry	4
3. Components	5
3.1. Kit contents	5
3.2. Materials to be supplied by user	5
3.3. Equipment needed	5
4. Description	6
4.1. Introduction	6
4.2. The basic principle	7
4.3. Product specifications	9
5. Protocol	11
5.1. Preparation of working solutions	11
5.2. Protocol for purification of oligonucleotides and small DNA fragments	11
6. Appendices	15
6.1. Related products	15
 Quick Reference Protocol Card	Back Cover
Tear off sheet containing protocols for the experienced user purifying oligonucleotides and small DNA fragments	

1. Legal

Product use restriction

The **illustra™ NAP™-10 Columns** and components have been designed, developed and sold **for research purposes only**. They are suitable **for *in vitro* use only**. No claim or representation is intended for their use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is the responsibility of the user to verify the use of the **illustra NAP-10 Columns** for a specific application, as the performance characteristics of this product have not been verified for any specific organism.

GE, imagination at work and GE monogram are trademarks of General Electric Company.

GFX, MicroSpin, AutoSeq, NAP, Rediprime, Sephadex, Ready-to-Go, PuReTaq, FideliTaq and *illustra* are trademarks of GE Healthcare companies.

ExoSAP-IT is a trademark of USB Corp.

Kathon is a trademark of Rohm and Haas Company.

© 2006–2007 General Electric Company-All rights reserved.

Previously published 2006. Date of revision Apr. 2007.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available upon request. Contact your GE Healthcare representative for the most current information (see back cover for contact details).

<http://www.gehealthcare.com/lifesciences>

GE Healthcare UK Limited.

Amersham Place, Little Chalfont,
Buckinghamshire. HP7 9NA UK.

2. Handling

2.1. Safety warnings and precautions

Warning: For research use only.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Suitable protective clothing such as laboratory overalls, safety glasses and gloves should be worn. Care should be taken to avoid contact with skin or eyes; if contact should occur, wash immediately with water (see Material Safety Data Sheet(s) and/or Safety Statement(s) for specific recommendations).

2.2. Storage conditions


Store at ambient temperature (4–30°C). **Do not freeze.**

2.3. Expiry

For expiry date please refer to outer packaging label.

3. Components

3.1. Kit contents

Identification	Pack size Cat. No.	20 purifications 17-0854-01	50 purifications 17-0854-02
	NAP™-10 Columns	20	50

Refer to the Certificate of Analysis for a complete list of kit components.

3.2. Materials to be supplied by user

Disposables:

DNase-free collection tubes

A suitable receptacle to catch waste buffer flow through

Chemicals:

Buffer 1

Any nuclease-free buffer is suitable, including water or Tris/EDTA (TE); both are available from GE Healthcare. It is important to use the same buffer in both the Column Equilibration and Elution steps.

20 ml **Buffer 1** per purification is ample.

3.3. Equipment needed

A column support

4. Description

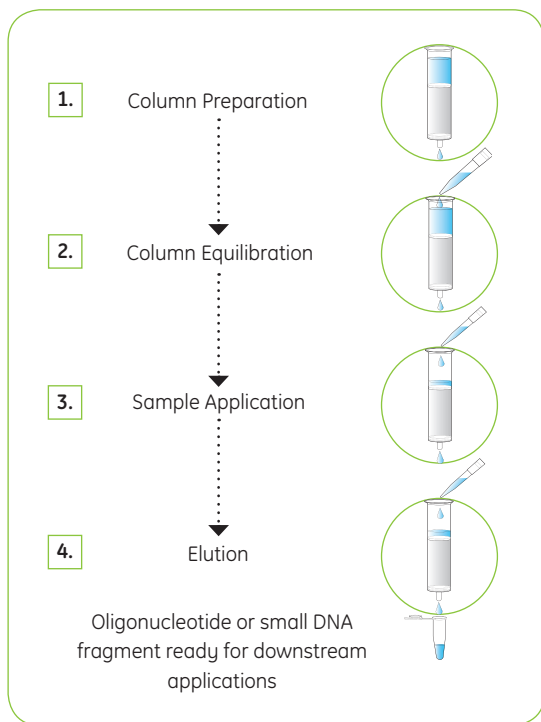
4.1. Introduction





illustra™ NAP Columns are disposable columns pre-packed with Sephadex™ G-25 DNA-grade resin and require only gravity to run. They allow DNA purification by the process of gel filtration. Molecules larger than the largest pores in the matrix are excluded from the matrix and elute first. Intermediate size molecules penetrate the matrix to varying extents, depending on their size. Penetration of the matrix retards progress through the column; very small molecules elute last. The volume required to elute these small molecules is dependent on the volume available both inside and outside the pores (i.e. the bed volume).

illustra NAP-10 Columns are designed for the rapid purification of DNA, by desalting and buffer exchange, and the removal of unincorporated nucleotides from end-labeled oligonucleotides. They can be used for any DNA greater than 10 bases in length and are therefore ideal for the purification of oligonucleotides or very small DNA fragments following synthesis or a labeling reaction. They will not remove or denature enzyme.

4.2. The basic principle

Use of **illustra NAP-10 Columns** involves the following steps:



Step	Comments	Component
1. Column Preparation	Excess storage buffer is allowed to flow through the column	
2. Column Equilibration	Column is equilibrated with buffer chosen by user (Buffer 1)	
3. Sample Application	Sample is applied to column	
4. Elution	Purified sample is eluted from the column with buffer chosen by user (Buffer 1)	

4.3. Product specifications

illustra NAP-10 Columns are recommended for the purification of oligonucleotides and small DNA fragments, the removal of salt and buffer exchange, and the removal of unincorporated nucleotides from end-labeling reactions. A summary of the product specifications are given in Table 1 below:

Table 1. illustra NAP-10 Column specifications

Sample Type:	Oligonucleotides and small DNA fragments
Principle	Gel filtration
Column matrix	Sephadex G-25 DNA grade
Column buffer	Distilled water containing 0.15 % Kathon™ CG/ICP Biocide as preservative
Maximum input sample volume	1 ml
Yield/recovery of DNA	> 90%
Purity of recovered DNA	Typically < 3 % salt contamination
Length of labeled DNA recovered	> 10 bp (N.B. There is no maximum length of oligonucleotide that can be purified)
Major subsequent applications	Polymerase chain reaction (PCR), sequencing, labeling
Column capacity (maximum amount of DNA that can be loaded onto column)	< 1 mg/ml Do not load a DNA solution of a concentration greater than 1 mg/ml. Higher concentrations reduce column resolution and give lower yield due to increased viscosity. Samples at a concentration greater than 1 mg/ml should be diluted with Buffer 1 prior to loading.

illustra NAP Columns are available in three sizes, NAP-5, NAP-10 and NAP-25 Columns. Selection of the correct column depends on the input sample volume: up to 0.5 ml (NAP-5 Columns), up to 1 ml (NAP-10 Columns) or up to 2.5 ml (NAP-25 Columns). Careful consideration should also be given to the volume requirements for the final purified sample. Table 2 below provides a selection guide to aid in choosing the appropriate NAP Column.

Table 2. illustra NAP Column selection guide

Column type	Input sample volume (ml)	Potential final purified sample volume (ml)
NAP-5	0.1	0.5
	0.25	0.7
	0.5 (maximum volume)	1.0
NAP-10	0.75	1.2
	1.0 (maximum volume)	1.5
NAP-25	1.5	2.5
	2.0	3.0
	2.5 (maximum volume)	3.5

If the sample volume is 100–150 μ l, and the final elution volume required is also 100–150 μ l, then use of GE Healthcare illustra MicroSpin™ G-25 Columns is recommended.

5. Protocol

Use of icons

The Key below describes the purpose of the icons used throughout the protocol booklet.



This icon is used to highlight particularly critical steps within the protocol that must be adhered to. If this advice is not followed it will have a detrimental impact on results.



This icon is used to highlight technical tips that will enhance the description of the step. These tips may indicate areas of flexibility in the protocol or give a recommendation to obtain optimum performance of the kit.

5.1. Preparation of working solutions

See section 3.2 and 3.3 for Materials and Equipment to be supplied by user.

Buffer 1

Any nuclease-free buffer is suitable, including water or Tris/EDTA (TE); both are available from GE Healthcare. It is important to use the same buffer in both the Column Equilibration and the Elution steps. 20 ml **Buffer 1** per purification is ample.

5.2. Protocol for purification of oligonucleotides and small DNA fragments



Note: Prior to commencing, ensure that the NAP-10 Columns have equilibrated to room temperature (20–25°C).

1. Column Preparation

- Remove the top and bottom caps from the NAP-10 Column and allow the excess liquid to flow through the column. Support the column over a suitable waste receptacle to catch buffer flow through.



Note: Not allowing the excess liquid to fully flow through the column will result in slow flow rates.

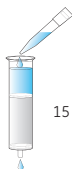
2. Column Equilibration

- Equilibrate the column with 15 ml of **Buffer 1**.



Note: This volume corresponds to 3 complete refills of the column. Any nuclease-free buffer is suitable, including water or Tris/EDTA (TE); both are available from GE Healthcare. It is important to use the same buffer in both the Column Equilibration and the Elution steps.

- Allow the **Buffer 1** to completely enter the gel bed by gravity flow. Do not apply positive pressure.



15 ml Buffer 1



3. Sample Application

- a. Add the sample to the column in a maximum volume of 1 ml. Allow the sample to enter the gel bed completely.



0.75-1 ml
sample



Note: If the sample volume is less than 1 ml, and you wish to elute your sample using the minimum volume of **Buffer 1** (see Table 3: Buffer volume guide, below), it is important not to adjust the sample volume to 1.0 ml with **Buffer 1** at this point.

For example: If 0.75 ml sample is loaded onto the column and allowed to enter the gel bed completely, and then 0.25 ml of **Buffer 1** is added to the column and allowed to enter the gel bed completely, the sample can be eluted using just 1.2 ml **Buffer 1** (as shown in Table 3 below).

Alternatively, if the sample volume is adjusted to 1 ml with **Buffer 1** and then added to the column, it would be necessary to elute with 1.5 ml of **Buffer 1** in order to obtain the maximum total yield of the sample (as shown in Table 3 below).

Table 3. Buffer volume guide

Column Type	Sample volume (ml)	Volume of Buffer 1 for Column Equilibration step	Volume of Buffer 1 for Elution step (ml)
NAP-10	0.75	0.25	1.2
	1.0	0	1.5
	(maximum volume)		



Note: Concentration of the DNA sample should be less than 1 mg/ml, as higher concentrations tend to reduce resolution and give lower yields due to increased viscosity.

- b. Where a sample volume of less than 1 ml has been loaded onto the column, load additional **Buffer 1** onto the column such that the combined volume of sample and buffer added equals 1 ml. Allow the **Buffer 1** to enter the gel bed completely.



Sample plus
Buffer 1 = 1 ml

4. Elution

- a. Place an appropriate size collection tube under the column.
- b. Elute the purified sample with an appropriate volume of **Buffer 1** (please refer to Table 3. Buffer volume guide).



1.2-1.5 ml
Buffer 1



Note: If you require a concentrated sample, collect 0.1 ml fractions as the sample elutes off the column. The concentration of the different fractions can be quantified using a spectrophotometer or by loading 5 μ l of each fraction onto an analytical gel (agarose or acrylamide).

- c. Store the purified sample at -20°C .



Up to 1.5 ml
purified sample

6. Appendix

6.1. Related products

A full range of Molecular Biology reagents can be found on the GE Healthcare web site and in the catalog.

<http://www.gehealthcare.com/illustra>

If you need further information, GE technical services are happy to assist (world-wide phone numbers can be found on the back cover)

Application	Product	Product code	Pack size
Buffer 1	TE Buffer, 50 ×	US75834	100 ml
	Water, nuclease-free	US70783	500 ml
Radioactive labeling	Rediprime™ II DNA Labeling System	RPN1633	30 reactions
	Nick Translation Kit	N5000	20 reactions
	5'-End Labeling Kit	RPN1509	20 reactions
Purification of oligonucleotides			
100–150 µl sample volume	illustra MicroSpin G-25 Columns	27-5325-01	50 purifications
Up to 0.5 ml sample volume	illustra NAP-5 Columns	17-0853-01	20 purifications
Up to 2.5 ml sample volume	illustra NAP-25 Columns	17-0852-01	20 purifications

Application	Product	Product code	Pack size
Purification of DNA from PCRs, agarose gel slices and enzyme reaction mixes	illustra GFX™ PCR DNA and Gel Band Purification Kit	28-9034-70	100 purifications
Preparation of PCR products for automated sequencing	ExoSAP-IT™	US78200	100 reactions
Dye terminator removal from automated sequencing reactions	illustra AutoSeq™ G-50 Columns	27-5340-01	50 purifications
Kits containing ready-to-use mix for PCR amplification	illustra Hot Start Master Mix	25-1500-01	100 reactions
	illustra PuReTaq™ Ready-To-Go™ PCR Beads	27-9557-01	96 reactions in 0.2 ml tubes/plate
	illustra PuReTaq Ready-To-Go PCR Beads	27-9557-02	5 × 96 reactions in 0.2 ml tubes/plate
	FideliTaq™ PCR Master Mix Plus (2 ×)	E71182	100 reactions

Application	Product	Product code	Pack size
	FideliTaq Master Mix Plus	E71183	100 reactions
Premixed nucleotides for PCR amplification	illustra DNA Polymerization Mix dNTP set (A, C, G, T) 20 mM each	28-4065-57	10 µmol
	illustra DNA Polymerization Mix dNTP set (A, C, G, T) 20 mM each	28-4065-58	40 µmol (4 × 10 µmol)
	illustra PCR Nucleotide Mix dNTP set (A, C, G, T) 25 mM each	28-4065-60	500 µl
	illustra PCR Nucleotide Mix dNTP set (A, C, G, T) 2 mM each	28-4065-62	1 ml

GE Healthcare offices:

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 Limited
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

GE Healthcare regional office contact numbers:

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: 1 800 463 5800
Fax: 1 800 567 1008

Central, East, & South East Europe
Tel: +43 1 972720
Fax: +43 1 97272 2750

Denmark
Tel: 45 16 2400
Fax: 45 16 2424

Eire
Tel: 01494 544000
Fax: 0044 1494 542010

Finland & Baltics
Tel: +358-(0)9-512 39 40
Fax: +358 (0)9 512 39 439

France
Tel: 01 6935 6700
Fax: 01 6941 9677

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 1990
Fax: 018 612 1910

Switzerland
Tel: 0848 8028 12
Fax: 0848 8028 13

UK
Tel: 0800 616928
Fax: 0800 616927

USA
Tel: +1 800 526 3593
Fax: +1 877 295 8102

<http://www.gehealthcare.com/illustra>

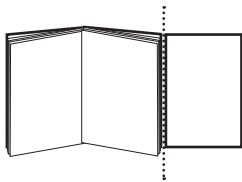
GE Healthcare UK Limited

Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA
UK



imagination at work

The next two pages are a
protocol card.
If required please add to the
back page as a tear off addition



If not then delete these three
pages.

Quick Reference Protocol Card

17-0854-01 (20 purifications)
17-0854-02 (50 purifications)

illustra™ NAP™-10 Columns

Protocol for purification of oligonucleotides and small DNA fragments, desalting and buffer exchange.

- Ensure suitable **Buffer 1** is available

⊕ : Add

1. Column preparation

- Remove top and bottom caps
- Allow excess liquid to drain by gravity flow

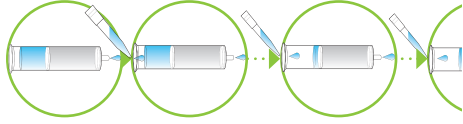
2. Column equilibration


- ⊕ 15 ml Buffer 1
- Allow to completely enter gel bed by gravity flow

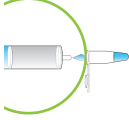
3. Sample application

- ⊕ 0.75–1 ml sample
- Allow to completely enter gel bed by gravity flow
- ⊕ Additional Buffer 1 as appropriate (see table below)
- Allow to completely enter gel bed by gravity flow

4. Elution



- Place an appropriate collection tube under column
-  Appropriate volume of Buffer 1
- Collect eluate by gravity flow
- Store purified sample at -20°C



Column Type	Sample volume (ml)	Volume of Buffer 1 for Column Equilibration step (ml)	Volume of Buffer 1 for Elution step (ml)
-------------	--------------------	---	--

NAP-10	0.75	0.25	1.2
1.0 (maximum volume)	0	1.5	1.5

GE, imagination at work and GE monogram are trademarks of General Electric Company. Illustra and NAP are trademarks of GE Healthcare Companies.

© 2007 General Electric Company.-All rights reserved. First published 2006.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available upon request. Contact your GE Healthcare representative for the most current information.

<http://www.gehealthcare.com/lifesciences>

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



imagination at work