cytiva

illustra NAP-10 Columns

Product booklet

Table of Contents

1 Introduction

Product codes

17085401 (20 purifications)

17085402 (50 purifications)

About

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

Important

Read these instructions carefully before using the products.

Intended use

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.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

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

Expiry

For expiry date please refer to outer packaging label.

2 Components

Kit contents

Identification	Pack size	20 purifications	50 purifications
	Product code	17-0854-01	17-0854-02
	NAP-10 Columns	20	50

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

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 Cytiva. It is important to use the same buffer in both the Column Equilibration and Elution steps. 20 mL Buffer 1 per purification is ample.

Equipment needed

A column support

3 Description

Background

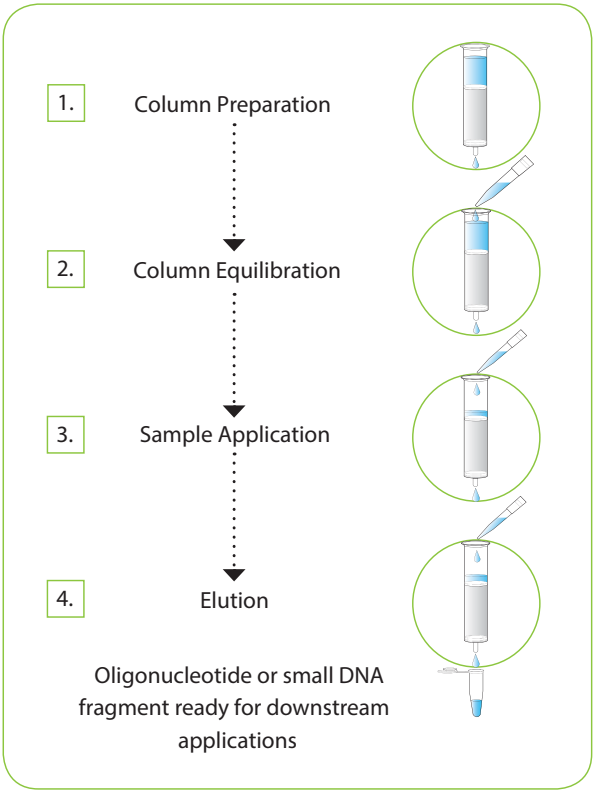
illustra NAP Columns are disposable columns pre-packed with Sephadex™ G-25 DNA-grade resin and require only gravity to run. They allow DNA purification by the process of gel filtration. Molecules larger than the largest pores in the matrix are excluded from the matrix and elute first. Intermediate size 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.

The basic principle

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

Illustration



Step procedure

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)	

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, on page 8*:

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 Column) 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, on page 9* 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 µL, and the final elution volume required is also 100–150 µL, then use of Cytiva illustra MicroSpin™ G-25 Columns is recommended.

4 Protocol

Preparation of working solutions

See *Materials to be supplied by user, on page 4* and *Equipment needed, on page 4* 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 Cytiva. 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.

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).*

Column Preparation

Step	Action
1	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	Proceed with the next step of the protocol.

Column Equilibration

Step	Action
1	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 Cytiva. It is important to use the same buffer in both the Column Equilibration and the Elution steps.*

Step	Action
2	Allow the Buffer 1 to completely enter the gel bed by gravity flow. Do not apply positive pressure.
3	Proceed with the next step of the protocol.

Sample Application

Step	Action
1	Add the sample to the column in a maximum volume of 1 ml. Allow the sample to enter the gel bed completely. ***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, on page 12), it is important not to adjust the sample volume to 1.0 mL with Buffer 1 at this point.* 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, on page 12*). 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, on page 12*).

Step	Action

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 (maximum volume)	0	1.5

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.

2 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

3 Proceed with the next step of the protocol.

Elution

Step	Action
1	Place an appropriate size collection tube under the column.

Step	Action
2	Elute the purified sample with an appropriate volume of Buffer 1 (please refer to *Table 3, on page 12*). ***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 µL of each fraction onto an analytical gel (agarose or acrylamide).*
3	Store the purified sample at -20°C.

5 Related products

A full range of Molecular Biology reagents can be found on *cytiva.com*.

cytiva.com/illustra

Application	Product	Product code	Pack size
Buffer 1	TE Buffer, 50 ×	US75834	100 mL
	Water, nucleasefree	US70783	500 mL
Radioactive labeling	Rediprime II DN 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	27532501	50 purifications

Application	Product	Product code	Pack size
Up to 0.5 mL sample volume	illustra NAP-5 Columns	17085301	20 purifications
Up to 2.5 mL sample volume	illustra NAP-25 Columns	17085201	20 purifications
Purification of DNA from PCRs, agarose gel slices and enzyme reaction mixes	illustra GFX™ PCR DNA and Gel Band Purification Kit	28903470	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	27534001	50 purifications
Kits containing ready-to-use mix for PCR amplification	illustra Hot Start Master Mix	25150001	100 reactions
	illustra PuReTaq Ready-To-Go™ PCR Beads	27955701	96 reactions in 0.2 mL tubes/ plate
	illustra PuReTaq Ready-To-Go PCR Beads	27955702	5 × 96 reactions in 0.2 mL tubes/ plate
	FideliTaq PCR Master Mix Plus (2 ×)	E71182	100 reactions
	FideliTaq Master Mix Plus	E71183	100 reactions

Application	Product	Product code	Pack size
Premixed nucleotides for PCR amplification	illustra DNA Polymerization Mix dNTP set (A, C, G, T) 20 mM each	28406557	10 µmol
	illustra DNA Polymerization Mix dNTP set (A, C, G, T) 20 mM each	28406558	40 µmol (4 × 10 µmol)
	illustra PCR Nucleotide Mix dNTP set (A, C, G, T) 25 mM each	28406560	500 µL
	illustra PCR Nucleotide Mix dNTP set (A, C, G, T) 2 mM each	28406562	1 mL

6 Quick reference protocol

Cue card

Quick Reference Protocol Card

illustra™ NAP™-10 Columns

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

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

- Ensure suitableBuffer 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

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For local office contact information, visit cytiva.com/contact

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