GE Healthcare

illustra
NICK Columns

Gravity flow columns for the removal of unincorporated radiolabeled nucleotides from DNA labeling reactions

Product booklet

Codes: 17-0855-01 (20 purifications)
17-0855-02 (50 purifications)

Page finder

1. Legal

Product use restriction

The **illustra™ NICK™ 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 NICK Columns** for a specific application, as the performance characteristics of this product have not been verified for any specific organism.

Previously published 2006. Date of revision Apr. 2007.

http://www.gehealthcare.com/lifesciences

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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 Statements(s) for specific recommendations).

2.2. Storage conditions

Store at ambient temperature (4°C–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 17-0855-01	50 purifications 17-0855-02
	illustra™ NICK™ 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. 5 ml **Buffer 1** per purification is ample.

Buffer 2

This buffer solution should be the same as the one in which your un-purified probe is solved. This may be the same as **Buffer 1**, but does not have to be. **Buffer 2** is used for the Column Equilibration, Sample Application and Elution steps and it is important to use the same buffer for each of these steps. 5 ml **Buffer 2** per purification is ample.

3.3. Equipment needed

A column support

4. Description

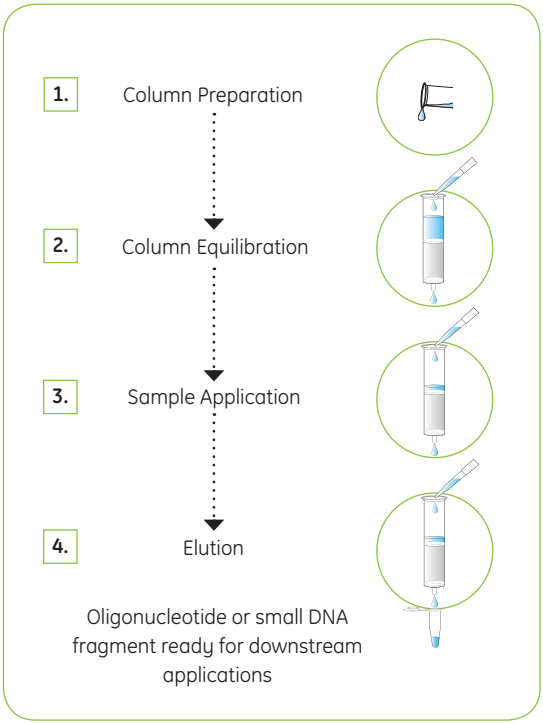
4.1. Introduction

illustra NICK Columns are single-use disposable columns pre-packed with Sephadex™ G-50 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 NICK Columns are designed for the rapid and convenient separation of nick-translated DNA from unincorporated ^{32}P-labeled nucleotides and similar separations. They can be used for any DNA greater than 20 bases in length. They will not remove or denature enzyme.

4.2. The basic principle

Use of **illustra NICK Columns** Columns involves the following steps:



Step	Comments	Component
1. Column Preparation	The storage buffer is poured away and the top of the column rinsed with buffer chosen by user (**Buffer 1**).	
2. Column Equilibration	Column is equilibrated with **Buffer 2** (buffer in which un-purified probe is solved).	
3. Sample Application	Sample is applied to column Additional **Buffer 2** is applied to the column.	
4. Elution	Purified sample is eluted from the column with **Buffer 2.**	

4.3. Product specifications

illustra NICK Columns are recommended for the removal of unincorporated radiolabeled nucleotides from DNA labeling reactions. A summary of the product specifications are given in Table 1 below:

Table 1. illustra NICK Column specifications

Sample Type:	**Oligonucleotides and small DNA fragments > 20 bases in length**
Principle	Gel filtration
Column matrix	Sephadex G-50 DNA grade
Input sample volume	1–100 µl
Column buffer	Distilled water containing 0.15 % Kathon™ CG/ICP Biocide as preservative
Yield/recovery of DNA	> 90%
Purity of recovered DNA	Typically < 0.2 % salt contamination
Length of labeled DNA recovered	> 20 bp (N.B. there is no maximum length of oligonucleotide that can be purified)
Volume of eluted purified sample	400 µl
Major subsequent applications	Hybridization
Gel bed dimensions	0.9 x 2.0 cm
Column capacity (maximum amount of DNA that can be loaded onto column)	100 µg 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.

For the quantitative removal of unincorporated labeled nucleotides from a DNA labeling reaction using spin-column chromatography, we recommend use of illustra ProbeQuant™ G-50 Micro Columns. These columns are suitable for purification of labeled DNA greater than 20 bases in length, and are also suitable for purification of biotinylated probes. Please note that these columns are supplied in 150 mM STE buffer containing 0.05 % Kathon.

For purification of labeled DNA less than 20 bases in length, we recommend use of illustra MicroSpin™ G-25 Columns. These columns are designed for the rapid purification of DNA, and will remove 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. They will not remove or denature enzyme. Please note that these columns are supplied in double-distilled water containing 0.05% Kathon.

Although tests have shown low levels of RNase activity for illustra NICK Columns, they are not specifically treated to be RNase free. Customers do regularly use illustra NICK columns for purification of RNA and are very satisfied. However, as the columns have not been tested for the absence of RNases we cannot guarantee that the RNA will not be degraded-but in principle it works well.

Biotinylated probes can be purified using illustra NICK Columns (as it is the size of the probe and not the modification that is relevant).

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 & 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. 5 ml **Buffer 1** per purification is ample.

Buffer 2

This buffer solution should be the same as the one in which your un-purified probe is solved. This may be the same as **Buffer 1**, but does not have to be. It is important to use the same buffer in the Column Equilibration, Sample Application and Elution steps. 5 ml **Buffer 2** per purification is ample.

5.2 Protocol for the removal of unincorporated radiolabeled nucleotides from DNA labeling reactions

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

1. Column Preparation

a. Remove the **top** cap from the NICK Column and pour off the excess storage liquid.

b. Rinse the top of the column once with **Buffer 1** and pour off the excess.

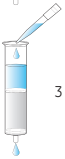
Rinse top of column with Buffer 1. Pour off excess.

Note: Any nuclease-free buffer is suitable, including water or Tris/EDTA (TE); both are available from GE Healthcare.

c. Remove the **bottom** cap. Support the column over a suitable waste receptacle.

2. Column Equilibration

a. Equilibrate the column with 3 ml of **Buffer 2.**



3 ml Buffer 2

Note: Buffer 2 should be the same as the one in which your un-purified probe is solved. This may be the same as **Buffer 1**, but does not have to be.

Note: This volume corresponds to 1 complete refill of the column.

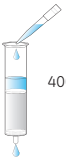
b. Allow **Buffer 2** to completely enter the gel bed by gravity flow. Do not apply positive pressure.

3. Sample Application

a. Add the sample to the top-centre of the resin column in any volume ranging from 1–100 µl. Allow the sample to enter the gel bed completely.

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. If necessary, dilute sample with **Buffer 1** prior to loading, but do not exceed maximum sample volume of 100 µl per column.

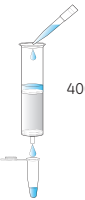
b. Add 400 µl **Buffer 2** to the column and allow to enter the gel bed completely.



4. Elution

a. Place an appropriately sized collection tube under the column.

b. Elute the purified sample with 400 µl **Buffer 2.**



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

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

A full range of Detection Products and available pack sizes can be found on the GE Healthcare web site and in the catalog http://www.gehealthcare.com/newhyperfilm

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
Blotting	Hybond™-N+ (82 mm)	RPN82B	50 discs
	Hybond-N+ (15 × 20 cm)	RPN1520B	10 sheets
	Hybond-NX (82 mm)	RPN82T	50 discs
	Hybond-NX (15 × 20 cm)	RPN1520T	10 sheets
	Hybond-N (82 mm)	RPN82N	50 discs
	Hybond-N (15 × 20 cm)	RPN1520N	10 sheets

Application	Product	Product code	Pack size
	Hybond-XL (82 mm)	RPN82S	50 discs
	Hybond-XL (15 × 20 cm)	RPN1520S	10 sheets
	Hybond blotting paper (20 × 20 cm)	RPN6101M	100 sheets
Radioactive labeling	Redivue™ nucleotides	AA0085	250 UCI – 1 MCI
	Rediprime™ II DNA Labeling System	RPN1633	30 reactions
	Ready-To-Go™ DNA Labeling Beads (-dCTP)	27-9240-01	1 kit
	Megaprime™ DNA Labeling System, dNTP	RPN1604	30 reactions
	Megaprime DNA Labeling System, dCTP	RPN1606	30 reactions
	Nick Translation Kit, dNTP	N5000	20 reactions
	5'-End Labeling Kit	RPN1509	20 reactions
Detection	Hyperfilm™ MP (18 × 24 cm)	28-9068-43	50 sheets
	Hyperfilm MP Enveloped (18 × 24 cm)	28-9068-50	50 sheets

Application	Product	Product code	Pack size
	Hypercassette™	RPN11642	1
Purification	illustra MicroSpin G-50 Columns	27-5330-01	50 purifications
	illustra MicroSpin G-25 Columns	27-5325-01	50 purifications
	illustra ProbeQuant G-50 MicroColumns	28-9034-08	50 purifications

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imagination at work

17-0855-01PL Rev B 06/2007

The next two pages are a protocol card.
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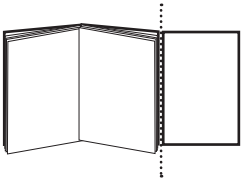

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Quick Reference Protocol Card

illustra™ NICK™ Columns

17-0855-01 (20 purifications)
17-0855-02 (50 purifications)

A. Protocol for the removal of unincorporated radiolabeled nucleotides from DNA labeling reactions

- Ensure suitable **Buffer 1** is available
- Ensure suitable **Buffer 2** is available

⊕ : Add

1. Column preparation

- Remove top cap
- Rinse top of column with Buffer 1 and pour off excess
- Remove bottom cap

2. Column equilibration

⊕ 3 ml Buffer 2

- Allow to completely enter gel bed by gravity flow

3. Sample application

⊕ 1–100 µl sample

- Allow to completely enter gel bed by gravity flow

⊕ 400 µl Buffer 2

- Allow to completely enter gel bed by gravity flow

4. Elution

- Place an appropriate collection tube under column
- 400 µl Buffer 2
- Collect eluate by gravity flow
- Store purified sample at -20°C

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17-0855-01PC Rev-B 06/2007