cytiva

illustra NICK Columns

Product booklet

Table of Contents

1 Introduction

Product codes

17085501 (20 purifications)

17085502 (50 purifications)

About

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

Important

Read these instructions carefully before using the products.

Intended use

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.

Safety

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

Storage

Store at ambient temperature (4°C–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	**17-0855-01**	**17-0855-02**
	illustra NICK 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. 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.

Equipment needed

A column support

3 Description

Background

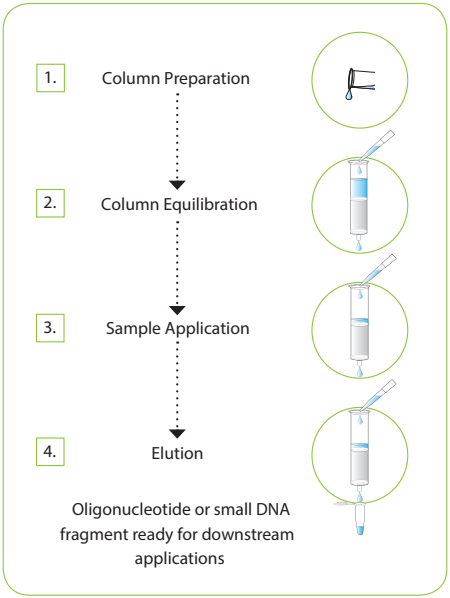
illustra NICK Columns are single-use disposable columns pre-packed with Sephadex™ G-50 DNA Grade resin and require only gravity to run. They allow DNA purification by the process of gel filtration. Molecules larger than the largest pores in the matrix are excluded from the matrix and elute first. Intermediate 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 32P-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.

The basic principle

Use of illustra NICK Columns involves the following steps:

Illustration



Step procedure

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

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, on page 8*

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).

4 Protocol

Preparation of working solutions

See section *Materials to be supplied by user, on page 4* and *Equipment needed, on page 5* 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 Cytiva. 5 mL Buffer 1 per purification is ample.

Buffer 2

This buffer solution should be the same as the one in which your unpurified 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.

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).*

Column Preparation

Step	Action
1	Remove the top cap from the NICK Column and pour off the excess storage liquid.
2	Rinse the top of the column once with Buffer 1 and pour off the excess. ***Note:*** *Any nuclease-free buffer is suitable, including water or Tris/EDTA (TE); both are available from Cytiva.*
3	Remove the bottom cap. Support the column over a suitable waste receptacle.

Step	Action
4	Proceed with the next part of the protocol.

Column Equilibration

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

Sample Application

Step	Action
1	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.*
2	Add 400 µL Buffer 2 to the column and allow to enter the gel bed completely.
3	Proceed with the next part of the protocol.

Elution

Step	Action
1	Place an appropriately sized collection tube under the column.
2	Elute the purified sample with 400 µL Buffer 2.
3	Store the purified sample at -20°C.

5 Related products

A full range of Molecular Biology reagents can be found on the Cytiva web site and in the catalog *cytiva.com/illustra*

A full range of Detection Products and available pack sizes can be found on *cytiva.com*.

Application	Product	Product code	Pack size
Buffer 1	TE Buffer, 50 ×	US75834	100 mL
	Water, nucleasefree	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
	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)	27924001	1 kit
	Megaprime DNA Labeling System, dNTP	RPN1604	30 reactions

Application	Product	Product code	Pack size
	Megaprime DNA Labeling System, dCTP	RPN1606	30 reactions
	Nick Translation Kit, dNTP	N5000	20 reactions
	5′-End Labeling Kit	RPN1509	20 reactions
Detection	Hyperfilm™ M (18 × 24 cm)	28906843	50 sheets
	Hyperfilm MP Enveloped (18 × 24 cm)	28906850	50 sheets
	Hypercassette	RPN11642	1
Purification	illustra MicroSpin G-50 Columns	27533001	50 purifications
	illustra MicroSpin G-25 Columns	27532501	50 purifications
	illustra ProbeQuant G-50 MicroColumns	28903408	50 purifications

6 Quick reference protocol card

Cue card

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

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