cytiva

illustra MicroSpin G-25 Columns

For the purification of oligonucleotides and small DNA fragments

Product booklet

Table of Contents

1 Introduction

Product code

27-5325-01 (50 purifications)

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

All kit components should be stored at room temperature (20°C to 25°C). **Do not freeze**.

Expiry

For expiry date please refer to outer packaging label.

2 Components

Kit contents

Identification	Component	Amount in kit
	illustra™ MicroSpin™ G-25 micro columns	50
	Collection tubes	50

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

Materials to be supplied by user

Disposables:

1.5 mL DNase-free microcentrifuge tubes

Equipment needed

- Microcentrifuge that accommodates 1.5 mL microcentrifuge tubes
- Vortex mixer (optional).

3 Description

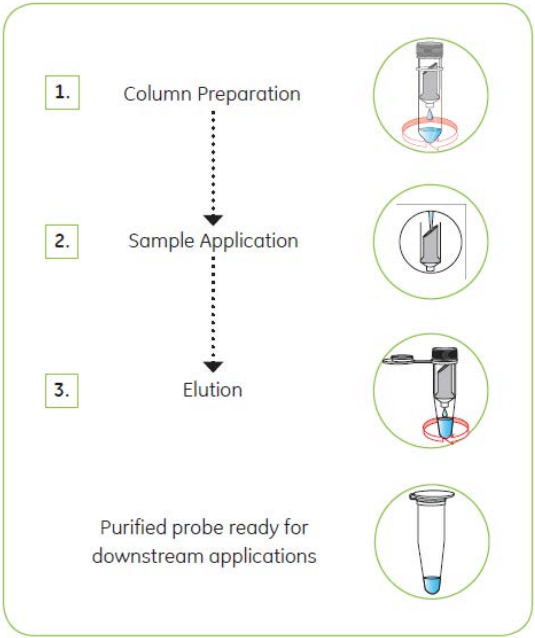
Introduction

illustra MicroSpin G-25 columns contain Sephadex™ G-25 DNA grade F resin. They allow DNA purification by the process of gel filtration. Molecules larger than the largest pores in the Sephadex are excluded from the gel and elute first. Intermediate size molecules penetrate the matrix to varying extents, depending on their size and the resin used. 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.

Gel filtration resins do not exhibit a fixed exclusion limit when used in a spin-column format. Exclusion limits of gel filtration resins are only meaningful in continuous flow processes where the molecules being purified have sufficient time to reach equilibrium between the time spent in the gel filtration medium and the time spent in the eluent stream. In spin-column chromatography, the observed exclusion properties that allow the product to pass through the gel while the smaller impurities are retained depends on experimental factors, such as: the resin used, sample volume, product size, and the g forces used in the purification process.

The basic principle

Use of illustra MicroSpin G-25 columns involves the following steps:



Step	Action
1	Column Preparation The resin is resuspended and excess storage buffer removed by centrifugation.

Step	Action
2	Sample Application The sample is applied to the column.
3	Elution Purified sample is eluted by centrifugation.

Product specifications

Table 1. illustra MicroSpin G-25 column specifications

Sample Type	Oligonucleotide or small DNA fragment
Principle	Gel filtration.
Column matrix	Sephadex G-25 DNA grade F.
Column storage buffer	Double distilled water containing 0.05% Kathon™ CG/ICP Biocide as preservative.
Input sample volume	100 to 150 µL when desalting oligonucleotides following de-protection in Ammonia. 25 to 50 µL when removing unincorporated labeled nucleotides from a oligonucleotide labeling reactions. 50 µL for buffer exchange or desalting of DNA.
Percent sample recovery	Variable-depends on input sample.
Length of labeled DNA recovered	> 10 bases (N.B. there is no maximum length of probe that can be purified).
Nuclease Testing	Column components are tested in nickase, single and double-stranded exonuclease and RNase assays.
Major subsequent applications	Dependent on input sample, but includes blotting, PCR and sequencing applications.

When to use an illustra MicroSpin G-25 column

The illustra MicroSpin G-25 column is designed for the rapid purification of DNA for use in a wide range of applications, including desalting, buffer exchange, and removal of labeled nucleotides from DNA labeling reactions. It is suitable for any DNA greater than 10 bases in length and is therefore ideal for the purification of oligonucleotides or very small DNA fragments following synthesis or a labeling reaction. It will not remove or denature enzyme. For guidelines to consider for use of illustra MicroSpin G-25 columns, please see *Guidelines for use of an illustra MicroSpin G-25 column, on page 13*

Cytiva provides a wide range of nucleic acid purification products, some of which may be better suited to your application. These products and the application for which they have been optimized are summarized in *Table 2, on page 9*. illustra AutoSeq G-50 columns and illustra ProbeQuant™ G-50 micro columns are provided pre-equilibrated in the optimal buffer for the application for which they are designed.

Table 2.

Application	Product	Product code	Pack size
PCR reaction and enzymatic DNA reaction purification 50 bp-10 kbp size range. Extraction of DNA from agarose gels.	illustra GFX™ PCR DNA and Gel Band Purification Kit	28903470 28903471	100 purifications 250 purifications
Dye terminator removal from automated sequencing reactions.	illustra AutoSeq G-50	27534001 27534002 27534003	50 purifications 250 purifications 1 000 purifications
Unincorporated labeled nucleotide removal from a DNA labeling reaction (> 20 mers).	illustra ProbeQuant G-50 MicroColumns	28903408	50 purifications
Purification of oligonucleotides following synthesis, buffer exchange and de-salting. Gravity format, 500 µL loading volume.	illustra NAP™-5 Columns	17085301	20 purifications

4 Protocol

Note: *Columns are NOT transferable between Cytiva kits, e.g., the composition of the MicroSpin G-25 columns is not the same as the composition of the MicroSpin G-50 columns.*

Notes and tips

Note: *A note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

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

See *Guidelines for use of an illustra MicroSpin G-25 column, on page 13* for guidelines to consider when purifying different sample types.

Protocol for purification of range samples

Column Preparation

Step	Action
1	Re-suspend the resin in the column by vortexing.
2	Loosen the cap one-quarter turn and twist off the bottom closure.
3	Place the column in the supplied Collection tube for support.
4	Spin for 1 minute at 735 × g. ***Tip:*** *See RPM calculation from RCF, on page 12.*

Step	Action
	Note: *Use columns immediately after preparation to avoid drying out of the resin. If the column resin appears dry, displaced or cracked after the first spin, this is usually indicative of overcentrifugation (too fast or too long). Re-hydrate the column with 250 µL of double distilled water, vortex and re-centrifuge, checking the settings. Spin speed can be reduced by 20% if necessary.* *Do not use the pulse button on the microcentrifuge as this may override the speed setting.*
5	Proceed immediately to Sample Application below.

Sample Application

Step	Action
1	Place the column into a fresh DNase-free 1.5 mL microcentrifuge tube (user supplied).
2	Slowly apply appropriate volume of sample to the top-center of the resin, being careful not to disturb the resin bed (see the Table below).

Table 3. Loading volumes for different sample types

Sample type	Loading volume (µL)
Oligonucleotides following de-protection in Ammonia	100 to 150
Oligonucleotide labeling reaction	25 to 50
Desalting or buffer exchange	50

Where sample volume exceeds that shown in table, use multiple columns, or consider use of NAP-5 Columns. Where sample volume is less than the minimum shown in the table, dilute the sample to improve product recovery (note concentration will be reduced).

Note: *The resin will have come away from the column slightly to form a pillar. It is essential that the sample being purified is applied slowly and is not allowed to run down the sides of the resin bed. Avoid touching the resin bed with the pipette tip.*

Elution

Step	Action
1	Spin for 2 minutes at 735 × g. The purified sample is collected in the bottom of the 1.5 mL microcentrifuge tube.
2	Store the purified probe at -20°C.

5 Appendices

RPM calculation from RCF

The appropriate centrifugation speed for a specific rotor can be calculated from the following formula:

RPM = 1 000 × √(RCF/1.12r)

Where RCF = relative centrifugal force, r = radius in mm measured from the center of the spindle to the bottom of the rotor bucket, and RPM = revolutions per minute.

For example, if an RCF of 735 × g is required using a rotor with a radius of 73 mm, the corresponding RPM would be 3 000.

The Table below shows appropriate RPM for various microcentrifuges.

Table 4. Appropriate RPM for an RCF of 735 × g

Microcentrifuge	Appropriate RPM for an RCF of 735 × g
Heraeus Biofuge 15	2 800
Beckman GS15R	2 100
Hettich Mikro 24-48	2 630
Hettich Mikro EBA12	2 700
Eppendorf Centrifuge 5415C	3 000
Eppendorf Centrifuge 5417C	2 700

Guidelines for use of an illustra MicroSpin G-25 column

illustra MicroSpin G-25 columns can be used for a wide variety of DNA purification applications. The DNA to be purified must be at least 10 bases in length. When using these columns, consider the following guidelines:

20 × rule

The best results will be obtained when the product being purified is at least 20 times larger than the largest impurity. If the difference in size is less than 20-fold, either purity or yield may be compromised.

Purity versus yield

In general, purity is inversely proportional to yield. Larger sample volumes will provide higher yield but lower purity, and vice-versa.

Non-specific binding

The non-specific binding exhibited by the illustra MicroSpin G-25 column is relatively insignificant, allowing purification of samples in the nanogram range. There will be a uniform proportional loss of sample which is due to the nature of spin column chromatography.

Retention

For a given sample volume, product retention is relative to molecular size. As the size of the product increases, its relative retention decreases.

Enzyme removal

For purification of DNA fragments 100 bp–10 kbp in length, following an enzymatic reaction, we recommend using the illustra GFX PCR DNA and Gel Band Purification Kit, as the enzyme will be removed during the spin column process. If using an illustra MicroSpin G-25 column, you must Phenol Chloroform extract prior to loading onto the column to ensure enzyme removal.

Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact Cytiva technical services. Alternatively log onto *cytiva.com/illustra*.

Table 5.

Possible causes	Suggestions
Poor sample purity	• Ensure the sample volume was within acceptable range prior to loading (see *Guidelines for use of an illustra MicroSpin G-25 column, on page 13*). • Ensure sample is CAREFULLY pipetted into centre of resin. Do not disturb the column. Do not allow the sample to run into the sides of the resin bed. • Use the column immediately after completing Column Preparation step. Do not allow the resin to become dried out or cracked.

Related products

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

A full range of Detection Products and available pack sizes can be found in the Cytiva catalog.

If you need further information, contact Cytiva technical services.

Table 6. Related products

Application	Product	Product code	Pack size
Removal of protein or enzyme	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

Application	Product	Product code	Pack size
	Hybond blottingpaper (20 × 20 cm)	RPN6101M	100 sheets
Radioactive labeling	Rediprime II DNA Labeling Systemx	RPN1633	30 reactions
	Ready-To-Go™ DNA Labeling Beads (-dCTP)	27924001	1 kit
	Megaprime DNA Labeling System, dNTP	RPN1604	30 reactions
	Megaprime DNA Labeling System, dCTP	RPN1606	30 reactions
	Nick Translation Kit, dNTP	N5500	20 reactions
	Nick Translation Kit, dCTP	N5000	20 reactions
	5′-End Labeling Kit	RPN1509	20 reactions
	Rapid-Hyb Buffer	RPN1635	125 mL
Detection	Hyperfilm™ MP (18 × 24 cm)	28906843	50 sheets
	Hyperfilm MPEnveloped (18 × 24 cm)	28906850	50 sheets
	Hypercassette	RPN11642	1
Purification of DNA probes and oligonucleotides	illustra ProbeQuant G-50 Micro Columns	28903408	50 purifications
	illustra NICK Columns	17085502	50 purifications
	illustra NAP-5 Columns	17085302	50 purifications
Purification of DNA from PCR, agarose gel bands and enzymes	illustra GFX PCR DNA & Gel Band Purification Kit	28903470	100 purifications
	illustra GFX 96 PCR Purification Kit	28903445	10 × 96 well plates
Preparation of PCR products for automated sequencing	ExoSAP-IT™	US78200	100 purifications

Application	Product	Product code	Pack size
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 purifications
	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
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 Que card

Quick Reference Protocol Card

illustra™ MicroSpin™ G-25 Columns

28-9034-08 (50 purifications)

Protocol for purification of a range of sample types

: Add : Spin

1. Column preparation

- Re-suspend the resin in the column by vortexing
- Loosen the cap one-quarter turn and twist off the bottom closure
- Place the column in the supplied Collection tube

1 minute 735 × g

2. Sample application

- Place the column into a fresh DNase-free 1.5 ml microcentrifuge tube (user supplied)

Sample to the top-center of the resin with care (see Table below)

3. Elution

2 minutes 735 × g

- Retain eluate
- Store the purified probe at -20°C

Table: Loading volumes for different sample types

Sample type	Loading volume (µl)
Oligonucleotides following de-protection in Ammonia	100–150
Oligonucleotide labeling reaction	25–50
De-salt or buffer exchange	50

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

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