cytiva

illustra MicroSpin Columns

For rapid buffer exchange, desalting and primer removal

Product Booklet

cytiva.com

27512001PL AH

Table of Contents

1 Introduction

Product codes

MicroSpin S-200 HR 27512001 (50 purifications)

MicroSpin S-300 HR 27513001 (50 purifications)

MicroSpin S-400 HR 27514001 (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

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

Storage

All kit components must be stored at 4%. Do not freeze.

Expiry

For expiry date refer to outer packaging label.

2 Components

Kit contents

Identification	Pack size Product code	50 purifications 27512001, 27513001 or 27514001
	illustra™ MicroSpin™ S-200, S-300 and S-400 HR 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 to be supplied by user

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

3 Description

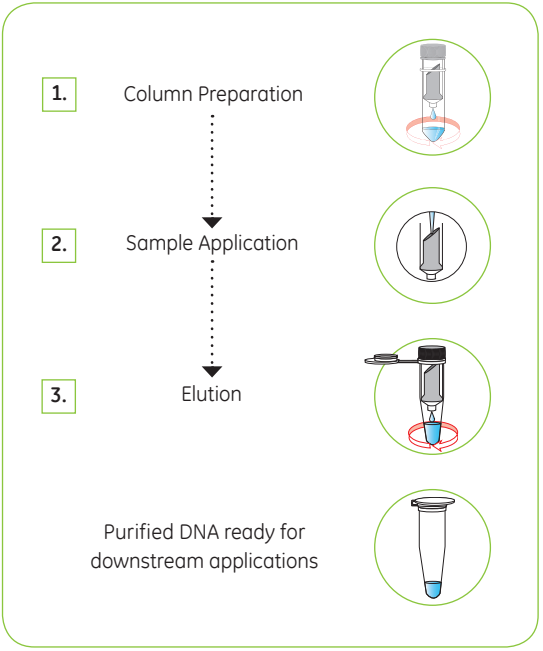
Introduction

illustra MicroSpin S-200, S-300 and S-400 HR columns contain Sephacryl™ resin of differing pore sizes. They allow DNA purification by the process of gel filtration. Molecules larger than the largest pores in the Sephacryl 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 S-200, S-300 and S-400 HR columns involves the following steps:



Step	Comments	Component
1. Column Preparation	The resin is resuspended and excess storage buffer removed by centrifugation.	illustra MicroSpin S-200, S-300 and S-400 HR  column
2. Sample Application	The sample is applied to the column.	
3. Elution	Purified sample is eluted by centrifugation.	

Product specifications

Table 1. illustra MicroSpin S-200, S-300 and S400 HR column specifications

Sample Type:	DNA radiolabeling reaction
Principle	Gel filtration
Column matrix	Sephacryl resin of appropriate pore size
Column storage buffer	TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.6)
Input sample volume	25 to 100 µL
Percent sample recovery	> 80%
Length of labeled DNA recovered	Variable-depends on input sample
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 cloning, PCR, blotting and sequencing applications

When to use an illustra MicroSpin S-200, S-300 or S-400 HR column

illustra MicroSpin S-200, S-300 and S-400 HR columns are designed for the rapid purification of DNA for a wide range of applications, including desalting, buffer exchange, removal of dye terminators from cycle sequencing reactions and removal of labeled nucleotides from DNA labeling reactions. Good product yield and purity is obtained with sample volumes from 25 to 100 µL, and from nanogram to milligram quantities of DNA. Enzymes will not be denatured or removed. For guidelines to consider for use of illustra MicroSpin S-200, S-300 and S-400 HR Columns, see *General guidelines for use of illustra MicroSpin S-200, S-300 and S-400 HR columns, on page 14* and *Column specific guidelines for use of illustra MicroSpin S-200, S-300 and S-400 HR Columns, on page 15*.

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 the table below. 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.

Application	Product	Product code	Pack size
PCR reaction and enyzymatic DNA reaction purification 50bp to 10kbp size range. Extraction of DNA from agarose gels	illustra GFX™ PCR DNA and Gel Band Purification Kit	28903470	100 purifications
		28903471	250 purifications
Dye terminator removal from automated sequencing reactions	illustra AutoSeq G-50	27534001 27534002 27534003	50 purifications 50purifications 50 purifications
Unincorporated labeled nucleotide removal from a DNA labeling reaction (> 20 mers)	illustra ProbeQuant G-50 Micro- Columns	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
Spin column format 150 µL loading volume	illustra MicroSpin G-25 Columns	27532501	50 purifications

4 Protocol

Note: *Columns are NOT transferable between Cytiva kits, e.g., the composition of the MicroSpin S-200, S-300 and S-400 HR Columns is not the same as the composition of the MicroSpin G-50 Columns.*

Use of icons

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

NOTICE
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.

Tip: *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.*

See section *General guidelines for use of illustra MicroSpin S-200, S-300 and S-400 HR columns, on page 14* and *Column specific guidelines for use of illustra MicroSpin S-200, S-300 and S-400 HR Columns, on page 15* for processing and analysis of these samples.

See *Materials to be supplied by user, on page 4* and *Equipment to be supplied by user, on page 4* for materials an equipment to be supplied by user.

Protocol for purification of a range of sample types

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. ***Note:*** *See RPM calculation from RCF, on page 13 for RPM calculation from RCF.* ***Tip:*** *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 TE buffer, 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 over-ride 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 25 to 100 µL sample to the topcenter of the resin, being careful not to disturb the resin bed (see *General guidelines for use of illustra MicroSpin S-200, S-300 and S-400 HR columns, on page 14* for volume of sample to add).

NOTICE

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 pipet 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	Cap the microcentrifuge tube.

Step	Action
3	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 2. 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

General guidelines for use of illustra MicroSpin S-200, S-300 and S-400 HR columns

illustra MicroSpin S-200, S-300 and S-400 HR columns can be used for a wide variety of DNA purification applications. When using these columns, consider the following guidelines.

20x 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. For any given volume, the larger the pore size of the resin, the greater the purity and lower the yield of the product which results. Gel filtration matrices with larger pore sizes (Sephacryl S-400>Sephacryl S-300>Sephacryl S-200) tend to retain more product than gel matrices with smaller pores.

Non-specific binding

The non-specific binding exhibited by the illustra MicroSpin S-200, S-300 and S-400 HR Columns 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.

Loading volumes

Load 25 to 100 µL onto a column for all applications.

For larger sample volumes, either use more than one column or reduce the sample volume by drying or precipitation. For smaller sample volumes, dilute the sample to improve product recovery. If the volume recommendations are followed, the yield of purified DNA is expected to be 50% to 90%.

Enzyme Removal

For purification of DNA fragments 50bp to 10kbp 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 S-200, S-300, or S-400 HR column, you must Phenol Chloroform extract prior to loading onto the column to make sure enzyme removal.

Column specific guidelines for use of illustra MicroSpin S-200, S-300 and S-400 HR Columns

For more specific column selection, a simplified applications guide is given in the table below.

Table 3. Column specific guidelines

Application	Notes	Recommended Column	Reaction volume
PCR reaction and enyzymatic DNA reaction purification (buffer exchange, desalting)	Will not remove enzyme[1]	S-200	25 to 50 µL
PCR reaction and enyzymatic DNA reaction purification (removal of excess primers prior to cloning)	Will not remove enzyme[1]	S-400	25 to 50 µL
PCR reaction and enyzymatic DNA reaction purification (removal of excess primers prior to other applications)	Will not remove enzyme[1]	S-300 S-400	25 to 50 µL 50 to 100 µL
Unincorporated labeled nucleotide removal from a DNA labeling reaction[2]		S-200 S-300 S-400	25 to 50 µL 50 to 75 µL 75 to 100 µL

[1] To remove enzyme from PCR or enzymatic reactions, use illustra GFX PCR DNA and Gel Band Purification Kit or Phenol Chloroform extract sample.

[2] DNA must be at least 100 bp in length for a good recovery. For DNA less than 100 bp in length, use illustra ProbeQuant G-50 Micro Columns.

For removal of unincorporated nucleotides from oligonucleotides, use illustra MicroSpin G-25 Columns.

Exceptions exist to these guides:

- Use illustra MicroSpin S-300 HR Columns for removal of primers from PCR reactions < 200 bp in length, regardless of the intended application.
- Use illustra MicroSpin S-400 HR Columns to remove primers that are greater than 24 bases in length, regardless of the size of PCR product.

Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information, contact Cytiva technical services. Telephone numbers are on the back page. Alternatively log onto *www.cytiva.com/illustra*.

Problem: Resin appears dry and cracked after Column Preparation step.

Possible causes	Suggestions
Poor sample purity	• Ensure the sample volume was within acceptable range prior to loading (see *General guidelines for use of illustra MicroSpin S-200, S-300 and S-400 HR columns, on page 14*). • Ensure sample is CAREFULLY pipetted into center 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 web site *www.cytiva.com/illustra*

A full range of Detection Products and available pack sizes can be found in the Cytiva catalog and on the web site *www.cytiva.com/hyperfilm*

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

Application	Product	Product code	Pack size
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

Application	Product	Product code	Pack size
	Hybond blotting paper (20 × 20 cm)	RPN6101M	100 sheets
Radioactive labeling	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
	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 MP Enveloped (18 × 24 cm)	28906850	50 sheets
	Hypercasette	RPN11642	1
Purification of DNA probes and oligonucleotides	illustra MicroSpin G-25 Columns	27532501	50 purifications
	illustra ProbeQuant G-50 Micro Columns	28903408	50 purifications

Application	Product	Product code	Pack size
	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 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 µmol
	illustra PCR Nucleotide Mix dNTP Set (A,C,G,T) 2 mM each	28406562	1 mL

6 Quick Reference Protocol Card

illustra MicroSpin S-200, S-300 and S-400 HR Columns 27512001, 27513001 or 27514001 (50 purification)

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)

25–100 µl of sample to the top-center of the resin with care

3. Elution

2 minutes at 735 × g.

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

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