GE Healthcare

illustra MicroSpin Columns

For rapid buffer exchange, desalting and primer removal

Product booklet

Codes:

MicroSpin S-200 HR	27-5120-01	(50 purifications)
MicroSpin S-300 HR	27-5130-01	(50 purifications)
MicroSpin S-400 HR	27-5140-01	(50 purifications)

Page finder

1. Legal

Product use restriction

The **illustra™ MicroSpin™ S-200, S-300 and S-400 HR 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 MicroSpin S-200, S-300 and S-400 HR Columns** for a specific application, as the performance characteristics of this product have not been verified for any specific organism.

Previously published July 2006.

http://www.gelifesciences.com

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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 Statement(s) for specific recommendations).

2.2. Storage

All kit components should be stored at 4°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.	50 purifications 27-5120-01, 27-5130-01 or 27-5140-01
	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.

3.2. Materials to be supplied by user

Disposables:
1.5 ml DNase-free microcentrifuge tubes.

3.3. Equipment to be supplied by user

Microcentrifuge that accommodates 1.5 ml microcentrifuge tubes
Vortex mixer (optional)

4. Description

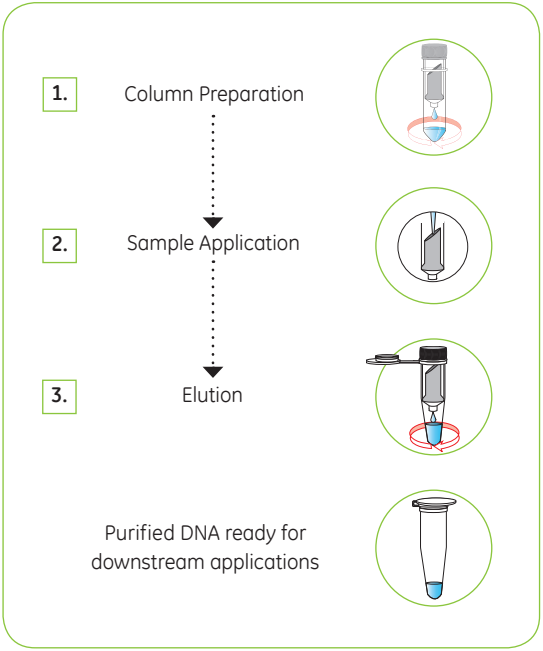
4.1. Introduction

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.

4.2. 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 re-suspended 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.	

4.3. 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, 1mM EDTA, pH 7.6)
Input sample volume	25–100 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

4.4. 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 25–100 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, please see sections 6.2 & 6.3.

GE Healthcare 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 1 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 **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	28-9034-70	100 purifications
		28-9034-71	250 purifications
Dye terminator removal from automated sequencing reactions	illustra AutoSeq G-50	27-5340-01	50 purifications
		27-5340-02	50 purifications
		27-5340-03	50 purifications
Unincorporated labeled nucleotide removal from a DNA labeling reaction (> 20 mers)	illustra ProbeQuant G-50 Micro-Columns	28-9034-08	50 purifications

Application	Product	Product code	Pack size
Purification of oligonucleotides following synthesis, buffer exchange and de-salting. Gravity format, 500 µl loading volume	illustra NAP™-5 Columns	17-0853-01	20 purifications
Spin column format 150 µl loading volume	illustra MicroSpin G-25 Columns	27-5325-01	50 purifications

5. Protocol

Note: Columns are NOT transferable between GE Healthcare 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.

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.

See section 6.2 & 6.3 for processing and analysis of these samples.

See section 3.2 and 3.3 for Materials & Equipment to be supplied by user.

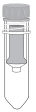
5.1. Protocol for purification of a range of sample types

1. Column Preparation

a. Re-suspend the resin in the column by vortexing.

b. Loosen the cap one-quarter turn and twist off the bottom closure.

c. Place the column in the supplied Collection tube for support.



d. Spin for 1 minute at 735 × g.

1 minute
735 × g

Note: See section 6.1 for RPM calculation from RCF.

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 over-centrifugation (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.

e. Proceed immediately to step 2 below.

2. Sample Application

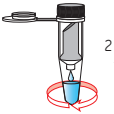
a. Place the column into a fresh DNase-free 1.5 ml microcentrifuge tube (user supplied).

b. Slowly apply 25–100 µl sample to the top-center of the resin, being careful not to disturb the resin bed (see section 6.2 for volume of sample to add).

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 pipet tip.

3. Elution

a. Spin for 2 minutes at 735 × g.
The purified sample is collected in the bottom of the 1.5 ml microcentrifuge tube.



b. Cap the microcentrifuge tube.

c. Store the purified probe at -20°C.

6. Appendices

6.1. RPM calculation from RCF

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

$RPM = 1\,000 \times \sqrt{(RCF/1.12r)}$

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.

Table 1 below shows appropriate RPM for various microcentrifuges.

Table 1: 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

6.2. 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:

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. 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 25–100 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 50–90%.

Enzyme Removal

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

6.3. 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 Table 3 below.

Table 3: Column specific guidelines

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

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

** 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.

6.4. Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact GE Healthcare technical services. Telephone numbers are on the back page. Alternatively log onto http://www.gelifesciences.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 section 6.2). • 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.

6 .6. Related products

A full range of Molecular Biology reagents can be found in the GE Healthcare catalog and on the web site http://www.gelifesciences.com/illustra

A full range of Detection Products and available pack sizes can be found in the GE Healthcare catalog and on the web site http://www.gelifesciences.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
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
Blotting ***Continued***	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)	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	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)	28-9068-43	50 sheets
	Hyperfilm MP Enveloped (18 × 24 cm)	28-9068-50	50 sheets
	Hypercassette™	RPN11642	1

Application	Product	Product code	Pack size
Purification of DNA probes and oligonucleotides	illustra MicroSpin G-25 Columns	27-5325-01	50 purifications
	illustra ProbeQuant G-50 Micro Columns	28-9034-08	50 purifications
	illustra NICK™ Columns	17-0855-02	50 purifications
	illustra NAP-5 Columns	17-0853-02	50 purifications
Purification of DNA from PCR, agarose gel bands and enzymes	illustra GFX PCR DNA & Gel Band Purification Kit	28-9034-70	100 purifications
	illustra GFX 96 PCR Purification Kit	28-9034-45	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	27-5340-01	50 purifications
Kits containing ready-to-use mix for PCR amplification	illustra Hot Start Master Mix	25-1500-01	100 reactions

Application	Product	Product code	Pack size
Kits containing ready-to-use mix for PCR amplification *Continued*	illustra PuReTaq™ Ready-To-Go PCR Beads	27-9557-01	96 reactions in 0.2 ml tubes/plate
	illustra PuReTaq Ready-To-Go PCR Beads	27-9557-02	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	28-4065-57	10 µmol
	illustra DNA Polymerization Mix dNTP Set (A,C,G,T) 20 mM each	28-4065-58	40 µmol (4 × 10 µmol)
	illustra PCR Nucleotide Mix dNTP Set (A,C,G,T) 25 mM each	28-4065-60	500 µmol

Application	Product	Product code	Pack size
Premixed nucleotides for PCR amplification *Continued*	illustra PCR Nucleotide Mix dNTP Set (A,C,G,T) 2 mM each	28-4065-62	1 ml

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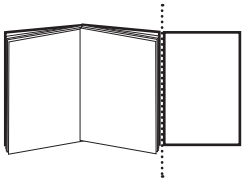

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Quick Reference Protocol Card

illustra™ MicroSpin™ S-200, S-300 and S-400 HR Columns

27-5120-01, 27-5130-01 or 27-5140-01 (50 purifications)

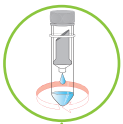
A. Protocol for purificationof a range of sample types

⊕ : 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

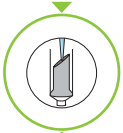
↻ 1 minute 735 × g



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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