GE Healthcare

illustra MicroSpin G-50 Columns

For rapid buffer exchange or desalting, dye terminator or primer removal and removal of labeled nucleotides from labeling reactions

Product booklet

Code: 27-5330-01 (50 purifications)
27-5330-02 (250 purifications)

Page finder

1. Legal

Product use restriction

The **illustra™ MicroSpin™ G-50 Columns** and components have been designed, developed and sold f**or 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 G-50 Columns** for a specific application, as the performance characteristics of this product have not been verified for any specific organism.

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 Statements(s) for specific recommendations).

2.2. Storage

All kit components should be stored at room temperature (20–25°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 27-5330-01	250 purifications 27-5330-02
	illustra™ MicroSpin™ G-50 columns	50	250
	Collection tubes	50	250

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 needed

Microcentrifuge that accomodates 1.5 ml microcentrifuge tubes
Vortex mixer

4. Description

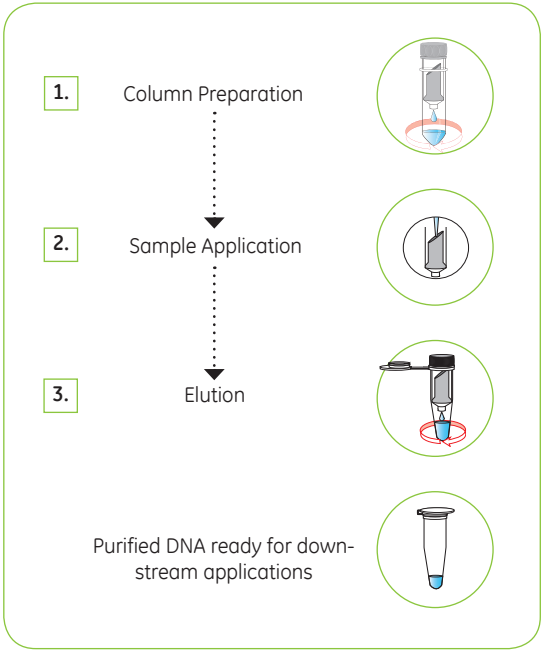
4.1. Introduction

illustra MicroSpin G-50 Columns contain Sephadex™ G-50 DNA-grade 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.

4.2. The basic principle

Use of **illustra MicroSpin G-50 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 G-50 column 
2. Sample Application	The sample is applied to the column.	
3. Elution	Purified sample is eluted by centrifugation.	

4.3. Product specifications

Sample Type:	Automated sequencing reactions
Principle	Gel filtration
Column matrix	Sephadex G-50 DNA grade F
Column storage buffer	TE buffer (10 mM Tris/HCl, 1mM EDTA) containing 0.05% Kathon™ CG/ICP Biocide as preservative.
Input sample volume	12–50 µl
Percent sample recovery	Variable-depends on input sample
Maximum column loading capacity	10 µg
Length of labeled DNA recovered	> 20 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 and sequencing applications.

4.4. When to use an illustra MicroSpin G-50 column

The illustra MicroSpin G-50 column is designed for the rapid purification of DNA for use in 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 12–50 µl. It is suitable for any DNA greater than 20 bases in length and will not remove or denature enzyme. For guidelines to consider for use of illustra MicroSpin G-50 Columns, please see section 6.2.

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.

Table 1: Optimized GE Healthcare products for specific applications

Application	Product	Product code	Pack size
PCR reaction and **enyzymatic 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 28-9034-71	100 purifications 250 purifications
Dye terminator removal from automated sequencing reactions	illustra AutoSeq G-50	27-5340-01 27-5340-02 27-5340-03	50 purifications 250 purifications 1 000 purifications
Unincorporated labeled nucleotide removal from a DNA labeling reaction (> 20 mers)	illustra ProbeQuant G-50 Micro Columns	28-9034-08	50 purifications
Purification of oligonucleotides following synthesis, buffer exchange and de-salting. Gravity format, 500 ml 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 G-50 Columns is not the same as the composition of the ProbeQuant G-50 Micro 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 3.2 and 3.3 for Materials & Equipment to be supplied by user.

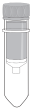
5.1. Protocol for purification of a range of sample types

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. For **removal of labeled nucleotides from DNA labeling reactions**, spin for 1 minute at **735 × g**. For **removal of dye terminators following cycle sequencing reactions,** spin for 1 minute at **2 000 × g**.

1 minute
735 × g
OR
1 minute
2 000 × 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 12–50 µl sample to the top-center of the resin, being careful not to disturb the resin bed.

12–50 µl
sample

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.

3. Elution

2 minutes
735 × g
OR
2 minutes
2 000 × g

a. For **removal of labeled nucleotides from DNA labeling reactions**, spin for 2 minutes at **735 × g**. For **removal of dye terminators following cycle sequencing reactions** spin for 2 minutes at **2 000 × 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.
E.g. 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 2 below shows appropriate RPM for various microcentrifuges.

Table 2: Appropriate RPM for an RCF of 735 × g and 2 000 × g

Microcentrifuge	**Appropriate RPM for an RCF of 735 × g**	**Appropriate RPM for an RCF of 2 000 × g**
Heraeus Biofuge 15	2 800	4 600
Beckman GS15R	2 100	3 600
Hettich Mikro 24–48	2 630	4 300
Hettich Mikro EBA12	2 700	4 400
Eppendorf Centrifuge 5415C	3 000	4 900
Eppendorf Centrifuge 5417C	2 700	4 400

6.2. Guidelines for use of an illustra MicroSpin G-50 column

illustra MicroSpin G-50 columns can be used for a wide variety of DNA purification applications. The DNA to be purified must be at least 20 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-50 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.

Loading volumes

Load 12–25 µl onto a column for dye terminator removal. We recommend use of illustra AutoSeq G-50 columns for this application as they have been optimized for sequencing reaction clean-up, and for salt-sensitive analyzers that utilize capillary loading.

Load 50 µl for removal of unincorporated labeled nucleotides from DNA labeling reactions. We recommend use of illustra ProbeQuant G-50 Micro-Columns for this application, especially when handling small (ng range) quantities of DNA. Recovery of DNA from illustra

MicroSpin G-50 columns is at least 10% less than that from illustra ProbeQuant G-50 micro-columns.

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.

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 G-50 column, you must Phenol Chloroform extract prior to loading onto the column to ensure enzyme removal.

6.3. 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: Poor sample purity

Possible cause	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.

6.4. 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 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
	Hybond blotting paper (20 × 20 cm)	RPN6101M	100 sheets
Radioactive labeling	Rediprime™ II DNA Labeling System	RPN1633	30 reactions

Application	Product	Product code	Pack size
Radioactive labeling ***Continued***	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

Application	Product	Product code	Pack size
Kits containing ready-to-use mix for PCR amplification	illustra Hot Start Master Mix	25-1500-01	100 reactions
	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 µl
	illustra PCR Nucleotide Mix dNTP Set (A,C,G,T) 2 mM each	28-4065-62	1 ml

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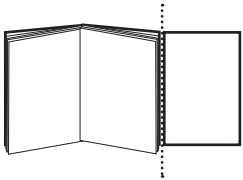

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Quick Reference Protocol Card

illustra™ MicroSpin™ G-50 Columns

27-5330-01 (50 purifications)
27-5330-02 (250 purifications)

A.Protocol for purification of a range of sample types

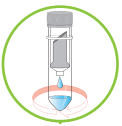
:Add :Spin

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 for removal of labeled nucleotides from DNA labeling reactions OR

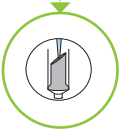
1 minute 2 000 x g for removal of dye terminators from cycle sequencing reactions



2. Sample application

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

12–50 µl of sample to the top-center of the resin with care



3. Elution

- 2 minutes 735 x g for removal of labeled nucleotides from DNA labeling reactions OR
- Spin 2 minutes 2 000 × g for removal of dye terminators from cycle sequencing reactions
- Retain eluate
- Store the purified sample at -20°C

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