GE Healthcare

illustra ProbeQuant G-50 Micro Columns

For the removal of unincorporated nucleotides from DNA labeling reactions

Product booklet

Code: 28-9034-08 (50 purifications)

Page finder

1. Legal

Product use restriction

The **illustra™ ProbeQuant™ G-50 Micro 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 ProbeQuant G-50 Micro Columns** for a specific application, as the performance characteristics of this product have not been verified for any specific organism.

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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

Store at ambient temperature (4–30°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 28-9034-08
	illustra ProbeQuant G-50 micro columns	50
	Probe buffer type 1 (Blue colored cap)	10 ml
	Collection tubes	50

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

GE Healthcare supplies a wide range of buffer types across the illustra nucleic acid purification and amplification range. The composition of each buffer has been optimized for each application and may vary between kits. Care must be taken to only use the buffers supplied in the particular kit you are using and not the buffers supplied in other illustra kits. Please ensure you use the correct type of Probe buffer for your purification.

3.2. Materials to be supplied by user

Disposables:
DNase-free 1.5 ml microcentrifuge tubes (screw caps are recommended when handling radioactivity).

Chemicals:
An excess of Probe buffer type 1 (150 mM STE buffer pH 8.0) is provided with the ProbeQuant G-50 micro columns. Should extra buffer be required, see section 6.5 for buffer composition.

3.3. Equipment needed

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

When measuring percent incorporation of radiolabel or determining counts per minute (cpm) per microliter (cpm/µl) of radiolabeled probe:
Scintillation vials or microcentrifuge tube holders
Scintillation counter
Additional DNase-free 1.5 ml microcentrifuge tubes (screw caps are recommended when handling radioactivity).

4. Description

4.1. Introduction

illustra ProbeQuant G-50 Micro Columns contain Sephadex™ G-50 DNA grade F. They purify DNA 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. 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, as with illustra ProbeQuant G-50 micro columns. Exclusion limits of gel filtration resins are only meaningful in continuous flow processes where the molecules being purified have sufficient time to reach an equilibrium between the time spent in the gel filtration medium and the time spend 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 during centrifugation.

The protocol provided with the illustra ProbeQuant G-50 micro columns has been optimized for the quantitative removal of unincorporated labeled nucleotides from a DNA labeling reaction only. GE Healthcare provides a wide range of nucleic acid purification products for other applications (see section 6.6 for more details).

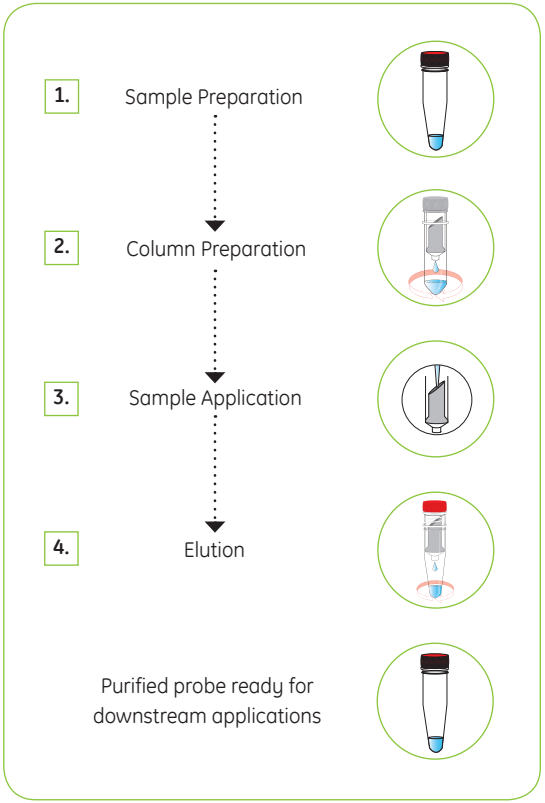
illustra ProbeQuant G-50 micro columns can be used simultaneously for both preparative and analytical applications (labeled DNA should be at least 20 bases in length). The columns can be used for the following:

- Purification of a DNA labeling reaction prior to applications such as hybridization.
- Determination of the cpm/µl of labeled probe.
- Measurement of the percent incorporation of a radionucleotide precursor into a labeled probe.

illustra ProbeQuant G-50 micro columns are ready-to-use and require less than 4 minutes from Sample Application to completion of the Elution step. These columns provide a pre-packed and pre-equilibrated alternative to Trichloroacetic Acid (TCA) precipitations. The sample elutes in 150 mM STE buffer, pH 8.0 (see section 6.5 for buffer composition).

4.2. The basic principle

Use of **illustra ProbeQuant G-50 Micro Columns** involves the following steps:



Step	Comments	Component
1. Sample Preparation	Radiolabeled sample is prepared and sample volume is made up to 50 µl with **Probe buffer type 1**.	**Probe buffer type 1**  **A range of labeling products are available from GE Healthcare (see section 6.6).**
2. Column Preparation	Sephadex G-50 resin is re-suspended and excess storage buffer is removed by centrifugation.	**illustra ProbeQuant G-50 micro column** 
3. Sample Application	Labeling reaction is applied to the center of the resin bed.	
4. Elution	Purified radiolabeled probe is eluted from the column, ready for downstream applications.	

4.3. Product specifications

Table 1. illustra ProbeQuant G-50 Micro Column specifications

Sample Type:	**DNA radiolabeling reaction**
Principle	Gel filtration
Column matrix	Sephadex G-50 DNA grade F
Column storage buffer	150 mM STE containing 0.15% Kathon™ CG/ICP Biocide as preservative.
Input sample volume	25–50 µl
Percent sample recovery	> 80%
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	Determination of the cpm/µl of labeled probe. Measurement of the percent incorporation of a radionucleotide precursor into a labeled probe. Hybridizations, such as Southern blots.

Although tests have shown low levels of RNase activity for these columns, they are not specifically treated to be RNase-free. Customers regularly use our ProbeQuant G-50 Micro Columns for purification of RNA and are very satisfied. However, as the columns have not been treated for absence of RNase we cannot guarantee that RNA will not be degraded-but in principle it works well.

Biotinylated probes can be purified using the illustra ProbeQuant G-50 Micro Columns, as it is the size of the probe and not the modification that is important for purification purposes.

For purification of labeled DNA less than 20 bases in length, we recommend use of illustra MicroSpin™ G-25 Columns. These columns are suitable for removal of unincorporated nucleotides from end-labeled oligonucleotides and small DNA fragments at least 10 bases long. Please note that illustra MicroSpin G-25 Columns are supplied in double distilled water containing 0.05% Kathon and for optimal results may need to be equilibrated in 150 mM STE buffer pH 8.0 before use.

If the use of a microcentrifuge with radiolabeled probes is an issue, consider use of the gravity flow system provided by illustra NICK™ Columns. Note that with illustra NICK Columns the final elution volume will be 400 µl.

5. Protocol

Note: Columns are NOT transferable between GE Healthcare kits, e.g., the composition of the ProbeQuant G-50 micro 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.

⟵ Determination of cpm/µl of labeled probe and percent incorporation of radiolabel. Where you see the arrow sign it is necessary to retain an aliquot of sample if you wish to determine cpm/µl of labeled probe or percent incorporation of radiolabel. 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 radiolabeled probe

1. Column Preparation

a. Perform the radiolabeling reaction according to instructions (A range of labeling products are available from GE Healthcare).

← Determination of cpm/µl of labeled probe and percent incorporation of radiolabel: Reserve 2 µl of the radiolabeling reaction for analysis at this stage. This is the TOTAL sample.

b. Adjust the volume of the sample to 50 µl with Probe buffer type 1 .

Note: If the sample volume is greater than 50 µl, use multiple columns, and apply a 50 µl aliquot to each column. Do not re-use columns. If more than 50 µl is loaded onto a column, unincorporated labeled nucleotides may elute off.

2. 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.

e. Proceed immediately to step 3.

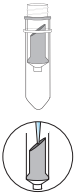
Note: See section 6.1 for RPM calculation from RCF.

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 **Probe buffer type 1**, 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

3. Sample Application

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

b. Slowly apply 50 µl of sample to the top-center of the resin, being careful not to disturb the resin bed.



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.

4. 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.

Determination of cpm/µl of labeled probe and percent incorporation of radiolabel: Reserve 2 µl of the purified sample for analysis at this stage. This is the COLUMN sample.

c. Proceed to section 6.2 to determine the cpm/µl of labeled probe or section 6.3 to determine the percent incorporation of the radiolabel.

d. 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. Determination of cpm/µl of labeled probe

a. Mix 2 µl of the "COLUMN" sample with 98 µl of **Probe buffer type 1** in a screw cap microcentrifuge tube.

b. Cap the tube and vortex gently to mix. Pulse spin in a microcentrifuge to collect the sample in the bottom of the microcentrifuge tube.

c. Dispense 2 × 50 µl aliquots of the diluted sample into duplicate screw capped microcentrifuge tubes.

d. Transfer each tube to a scintillation vial or suitable tube holder for placement into the scintillation counter. Alternatively, the samples may be added directly to scintillation vials containing scintillant; cap and invert to mix.

e. Count the samples using an appropriate program.

Note: ^{32}P-labeled samples may be counted using a 1 minute Cerenkov counting program if no scintillant is used, or a standard counting program if scintillant is used.

f. Add the counts per minute obtained for the duplicate samples and divide by 2. This value is the average cpm or cpm/µl of the labeled probe.

Note: To determine the total cpm incorporated into the purified sample, multiply cpm/µl by the volume of the sample recovered from the column.

6.3. Determination of percent incorporation of radiolabel

a. Mix 2 µl of the "COLUMN" sample with 98 µl of **Probe buffer type 1**  .

b. Mix 2 µl of the "TOTAL" sample with 98 µl of **Probe buffer type 1**  .

c. Cap the tubes and vortex gently to mix. Collect sample in bottom of microcentrifuge tube by pulse centrifugation.

d. Dispense 2 × 50 µl aliquots of diluted COLUMN and 2 × 50 µl aliquots of diluted TOTAL sample into 4 microcentrifuge tubes.

e. Transfer each tube to a scintillation vial or suitable tube holder for placement into the scintillation counter. Alternatively, the samples may be added directly to scintillation vials containing scintillant; cap and invert to mix.

f. Count the samples using an appropriate program.

Note: ^{32}P-labeled samples may be counted using a 1 minute Cerenkov counting program if no scintillant is used, or a standard counting program if scintillant is used.

g. Add the cpm obtained for diluted "COLUMN" samples and divide by 2 to obtain the average. Add the cpm obtained for diluted TOTAL samples and divide by 2 to obtain the average.

h. Percent label incorporation may be calculated using the formula:

$$\% \text{ label incorporartion} = \frac{\text{average cpm for "COLUMN" sample}}{\text{average cpm for "TOTAL" sample}} \times 100$$

Note: Percent incorporation values obtained using this product may vary from values previously obtained by standard TCA analysis or ethanol precipitation. This product is not designed to replace these methods, but is meant to offer a fast alternative to standard techniques.

6.4. Troubleshooting guide

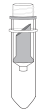
This guide may be helpful in the first instance, however if problems persist or for further information please contact GE Healthcare technical services. Telephone numbers are on the back page. Alternatively log onto http://www.gelifesciences.com/illustra.

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

Possible causes	Suggestions
Centrifugation steps too long or too fast.	• Ensure that columns are spun at the correct speed and for the appropriate time. For conversion of RCF to RPM see section 6.1. • If problems persist, reduce spin speed by 20%.

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

Possible causes	Suggestions
Centrifugation steps too long or too fast. Continued	• If column is considered too dry, or centrifugation is known to have been too fast or too long, add 250 µl Probe buffer type 1 to the column and re-centrifuge using the correct settings.
Use of the pulse button on the microcentrifuge.	• Do not use the pulse button on the microcentrifuge as this could over-ride the speed setting.
There is nothing wrong; this is normal.	• After centrifugation to remove the storage buffer, the resin will appear dry and will pull away from the sides of the column. However, excessive cracking should always be investigated.



Problem: Final sample volume is greater than 50 µl

Possible causes	Suggestions
Spin for Column Preparation step too short or too slow.	• Ensure that columns are spun at the correct speed and for the appropriate time. For conversion of RCF to RPM see section 6.1.
Spin for Elution step too long or too fast.	• Ensure that columns are spun at the correct speed and for the appropriate time. For conversion of RCF to RPM see section 6.1. • If problems persist, reduce centrifugation step by 20%.
Use of the pulse button on the microcentrifuge.	• Do not use the pulse button on the microcentrifuge as this could over-ride the speed setting.

Problem: Final sample volume is much less than 50 µl

Possible causes	Suggestions
Spin for Column Preparation step too fast or too long and/or Elution step too slow or too short.	• Ensure that columns are spun at the correct speed and for the appropriate time. For conversion of RCF to RPM see section 6.1. • If problems persist, reduce centrifugation speed by 20%. • If column is considered too dry, or centrifugation is known to have been too fast or too long, add 250 µl Probe buffer type 1 to the column and re-centrifuge using the correct settings.
Use of the pulse button on the microcentrifuge when spinning ProbeQuant G-50 micro columns.	• Do not use the pulse button on the microcentrifuge as this could over-ride the speed setting.

Problem: Unincorporated nucleotides remain in purified sample

Possible causes	Suggestions
Column not used immediately after preparation, allowing the resin to dry and crack. Sample will run through the cracks.	• Use column immediately after preparation.
Sample not pipetted onto top of resin bed, but allowed to run down the sides.	• CAREFULLY pipet the sample onto the top center of the resin bed.

Problem: Unincorporated nucleotides remain in purified sample
Continued

Possible causes	Suggestions
Sample volume added greater than 50 µl.	• If sample to be purified is greater than 50 µl, use multiple columns. purified
Spin for Elution step too long or too fast	• Ensure that columns are spun at the correct speed and for the appropriate time. For conversion of RCF to RPM see section 6.1. • If problems persist, reduce centrifugation speed by 20%.

6.5 Buffer composition

Use nuclease free water and sterile plasticware for buffer preparation. Filter sterilize before use.

To prepare 50 ml of additional **Probe buffer type 1**, dissolve 0.438 g of NaCl in 40 ml of TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) with stirring. Titrate the solution to pH 8.0 using dilute HCl or NaOH, as required. Transfer the solution to a 50 ml graduated cylinder and adjust the volume to 50 ml with TE buffer, pH 8.0.

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
	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

Application	Product	Product code	Pack size
Radioactive labeling ***Continued***	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
Purification of DNA probes and oligonucleotides	illustra MicroSpin G-50 Columns	27-5330-01	50 purifications
	illustra MicroSpin G-25 Columns	27-5325-01	50 purifications
	illustra ProbeQuant G-50 Micro Columns	28-9034-08	50 purifications

Application	Product	Product code	Pack size
Purification of DNA probes and oligonucleotides ***Continued***	illustra NICK Columns	17-0855-02	50 purifications
	illustra NAP™-5 Columns	17-0853-02	50 purifications

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Quick Reference Protocol Card

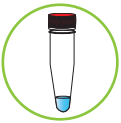
illustra™ ProbeQuant™ G-50 Micro Columns 28-9034-08 (50 purifications)

A. Protocol for purification of radiolabeled probe

⊕ : Add ↻ : Spin

1. Sample Preparation

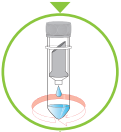
- Perform the radiolabeling reaction according to instructions
- Adjust the volume of the sample to 50 µl with Probe Buffer type 1



2. 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

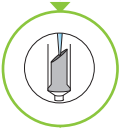
↻ 1 minute 735 × g



3. Sample Application

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

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



4. Elution

2 minutes at 735 × g

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

Quick Reference Protocol Card

B. Determination of cpm/µl of labeled probe and percent incorporation of radiolabel

- Mix 2 µl COLUMN sample with 98 µl Probe buffer type 1
- Vortex. Pulse centrifuge
- Dispense 2 × 50 µl aliquots into suitable tubes or vials
- Count as appropriate
- Determine mean cpm of the COLUMN sample. This is the cpm/µl of radiolabeled probe

- Mix 2 µl of the TOTAL sample with 98 µl Probe buffer type 1
- Vortex. Pulse centrifuge
- Dispense 2 × 50 µl aliquots into scintillation vials
- Count as appropriate
- Determine mean cpm of the TOTAL sample.
  Use formula below:

$$\%\ \text{label incorporation} = \frac{\text{average cpm for ''COLUMN'' sample}}{\text{average cpm for ''TOTAL'' sample}} \times 100$$

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