Quick Spin High Capacity Columns

G-50 Sephadex Columns for DNA Purification and Desalting (Exclusion limit: <72 base pairs)

Cat. No. 03 117 928 001 20 columns

Version 06
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Store at at +2 to +8°C

1. What this Product Does

Contents

Pre-packed, pre-swollen, columns (3.0 ml bed volume). Suspension of Sephadex G-50 in TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA).

Storage and Stability

The columns are stable through the control date (one year from date of manufacture) when stored in the refrigerator at +2 to +8°C. **Do not freeze**.

To avoid contamination of the columns and collection tubes, leave them in the storage bag until just before use.

Appearance

Each column contains a 3.0 ml bed volume of pre-swollen matrix. Each column unit consists of a ready-to-use column, a 1.0 ml polypropylene collection tube for collecting the purified DNA sample, and a 10 ml polypropylene conical adaptor tube. Column units are packaged in resealable storage bags to prevent contamination

Application

Quick Spin High Capacity columns are ready-to-use disposable column units designed to quickly and efficiently remove unincorporated nucleotides and oligonucleotides from DNA labeled by nick translation, end-labeling, polymerization reactions, and other labeling techniques. These Quick Spin High Capacity columns have been optimized for sample volumes ranging from 100 µl to 500 µl.
Quick Spin columns are designed for use in low-speed, swinging-bucket centrifuges.

Product Description

Quick Spin High Capacity Columns are produced with material shown to have high recovery of DNA (≥90%). All the tedious, time-consuming steps involved in preparing columns have already been performed. The columns are pre-swollen, pre-packed, pre-spun, and quality tested to ensure

- maximal retention of unincorporated nucleotides (≥98%), and
- absence of DNase contamination.

2. How to Use this Product

2.1 Before you Begin

General Handling Recommendations

Instructions for using the Quick Spin High Capacity columns to purify DNA are given below. If you are working with radiolabeled DNA, we recommend that you follow appropriate safeguards such as wearing protective gloves and safety glasses and using lucite shielding. See ref. 1 for a thorough treatment of the precautions to follow when handling radioactive compounds.

Take appropriate precautions when working with any type of hazardous materials. We recommend that you use a double containment system (*e.g.,* place the Quick Spin column/collection tube in a conical plastic carrier tube) whenever you work with radioactive reagents or other hazardous materials. We also recommend verifying that the filter is properly seated in the column (*i.e.,* present and not tilted so as to allow Sephadex to pass through).

⚠ Please read the entire "Procedure" section (including the "Notes on Centrifugation") before proceeding.

Procedure

1. Remove the column from the resealable bag and gently invert it several times to resuspend the separation medium.
2. Remove the top cap from the column, then remove the bottom tip. Placing the column in a 10 ml adapter tube, allow the buffer to briefly drain by gravity.
   The top cap must be removed first to avoid creating a vacuum and uneven flow of the column buffer.
3. Centrifuge the column in the adapter tube at 1,100 × *g* for 4 min (See "Notes on Centrifugation" listed below).
4. Carefully remove the column from the adapter tube. Discard the eluted buffer, shaking the inverted adapter tube to remove any residual buffer.
5. Keeping the column in an upright position, very carefully apply the DNA sample (in 100 to 300 µl) to the center of the column bed.
   Avoid applying the sample to the sides of the column; if this occurs, nucleotides flow around the separation medium and are not retained.
   When applying more than 300 µl of sample to a column, apply it in two aliquots to aid absorption.
   The sample volume applied can affect nucleotide retention and DNA recovery; review Tables 1 and 2 before proceeding.

Table 1. Effect of sample volume on retention of 1 mmol of nucleoside triphosphate.

		% nucleotide retention
Sample	100 µl	>99%
Volume	250 µl	>98%
Applied	500 µl #	>90%

Table 2. Effect of sample volume on DNA recovery.

		% recovery of 1 µg DNA	% recovery of 10 µg DNA
Sample	100 µl	60 – 70%	>99%
Volume	250 µl	>85%	>90%
Applied	500 µl #	>85%	>98%

Applied in two 250 µl aliquots

6. Place a 1.5 ml collection tube in the bottom of the 10 ml adapter tube.
7. Keeping the column in an upright position, carefully place the column in the adapter tube so that the bottom tip of the column hangs just inside the 1.5 ml collection tube.
   Maintaining the column in an upright position is very important, especially after centrifugation. Tipping the column causes back-flow of the DNA sample, resulting in reduced DNA recovery.
8. Centrifuge for 6 min at 1,100 × *g*.

❾	Carefully remove the column from the adapter tube and discard it in a designated (radioactive) waste container.
❿	Carefully remove (with oversized forceps) the 1.5 ml collection tube from the adapter tube, or carefully pipet the eluate from the collection tube into another storage vessel. Save this eluate. This contains your purified DNA sample.

Notes on Centrifugation

- The rpm required to obtain a relative centrifugal force of 1,100 × *g* will vary according to the centrifuge and rotor being used. Make certain that the centrifuge is accurately calibrated. Information from the centrifuge manufacturer will allow you to convert the rpm to g-force.
- Quick Spin High Capacity columns can be centrifuged in most tabletop/clinical centrifuges and most low-speed floor model centrifuges that use swinging-bucket rotors.

  Be sure to use a swinging-bucket rotor rather than a fixed-angle rotor. In a swinging bucket rotor, the sleeves swing out as the speed of the centrifuge increases so that the force on the tube is always straight through the center instead of at an angle. In a fixed-angle rotor, the sample is likely to slide down the sides of the tube instead of flowing through the separation medium. This results in poor retention of nucleotides and decreased recovery of sample.

3. Additional Information on this Product

Nucleotide Retention

Each lot of Quick Spin High Capacity Columns is tested to ensure that nucleotides are retained by the matrix., using a dye-based assay. When potassium ferricyanide dye (MW = 329.3) is loaded onto the Quick Spin High Capacity column and processed according to the Instructions for Use, greater than 98% of the dye is retained by the column (see table 1).

Absence of DNase Contamination

Each lot of Quick Spin High Capacity columns is tested to ensure the absence of DNase activity. One microgram of lambda DNA is incubated in 50 µl of Quick Spin column eluate in the presence of 1 mM $MgCl_2$ for 6 hours at +37°C. After incubation, the DNA samples are subjected to electrophoresis in a 1% agarose gel and stained with ethidium bromide. No degradation products are observed.

3.1 References

1 Zoon, R.A. (1987) *Methods in Enzymology* **152**, 25-29.

2 Dubose, R.F. and Hartl, D. L. (1990) *BioTechniques* **8**, 271.

4. Supplementary Information

Changes to Previous Version

Editorial changes.

Text Conventions

To make information consistent and understandable, the following text conventions are used in this document:

Text Convention	Use
Numbered instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed.
Paragraph §	Denotes a product available in the US only.

Symbols

Symbols are used in this document to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Other Quick Spin Products

Roche Diagnostics supplies columns optimized for biotinylated DNA and RNA purification, radiolabeled DNA and RNA purification, size-fractionation of cDNA, and removal of PCR primers. All columns have been treated and tested to ensure the absence of DNase contamination. Quick Spin columns for RNA purification have also been specially treated and function-tested for the absence of RNase contaminants. In addition, all columns have been tested to ensure maximal recovery of DNA or RNA, and retention of nucleotides.

- **Quick Spin Columns (G-25 and G-50) for radiolabeled DNA purification**: G-25 columns have an exclusion limit of 10 bases and are available in STE (Cat. Nos. 11 273 922 001, 11 273 949 001) or TE (Cat. No. 11 522 990 001§) buffer; G-50 columns have an exclusion limit of 72 bases and are available in STE (Cat. Nos. 11 273 965 001, 11 273 973 001) or TE (Cat. No. 11 523 023 001§) buffer.
- **Quick Spin Columns (G-25 and G-50) for radiolabeled RNA purification**: G-25 columns have an exclusion limit of 10 bases (Cat. No. 11 273 990 001); G-50 columns have an exclusion limit of 72 bases (Cat. No. 11 274 015 001).
- **Quick Spin High Capacity Columns (G-50) for DNA purification** have an exclusion limit of 72 bases and are optimized for sample volumes ranging from 100 µl to 500 µl (Cat. No. 03 117 928 001§).
- **Quick Spin Columns (G-50) for biotinylated RNA purification** (Cat. No. 03 117 464 001§) have an exclusion limit of 72 bases.
- **Mini Quick Spin Columns**: Microfuge-compatible G-25 columns in STE buffer are available for oligonucleotide purification (Cat. No. 11 814 397 001); microfuge-compatible G-50 columns in STE are available for radiolabeled DNA (Cat. No. 11 814 419 001) and RNA (Cat. No. 11 814 427 001) purification.

Roche Diagnostics also supplies the mRNA Capture Kit, and a series of HIGH PURE columns for purifying nucleic acids from various prokaryotic and eukaryotic sources. Contact your local representative for more information.

Trademarks

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List of biochemical reagent products

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To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany