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mini Quick Spin Columns

Version 07

Content version: February 2013

Ready-to-use, microcentrifuge-compatible chromatography columns for quick and efficient purification of nucleic acids from labeling reactions

Cat. No. 11 814 419 001	50 mini Quick Spin DNA Columns
Cat. No. 11 814 427 001	50 mini Quick Spin RNA Columns
Cat. No. 11 814 397 001	50 mini Quick Spin Oligo Columns

Store columns at +2 to +8°C. Do not freeze.

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1. What this Product Does

Contents

Content	Cat. No.
50 mini Quick Spin DNA Columns	11 814 419 001
50 mini Quick Spin RNA Columns	11 814 427 001
50 mini Quick Spin Oligo Columns	11 814 397 001

mini Quick Spin Columns are prepared by suspending Sephadex G-25 (for Oligo columns) or Sephadex G-50 (for DNA or RNA columns) matrix in 1× STE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA; 100 mM NaCl). The suspended Sephadex matrix is then autoclaved and packed into irradiated columns under aseptic conditions.
mini Quick Spin RNA Columns are treated with diethylpyrocarbonate (DEPC) before filling to ensure RNase-free columns.
Columns are packaged in a "zippered" storage bag.

Stability

- Columns are stable when stored at +2 to +8°C.
- **Do not freeze columns.**

Additional Equipment and Reagents Required

- Variable speed microcentrifuge
- 1.5 ml sterile microcentrifuge tubes (2 per column)

Application

The mini Quick Spin columns are designed for quick and complete removal of unincorporated nucleotides (*e.g.,* radionucleotides or fluorescent dye-labeled dideoxy terminators) from labeled nucleic acids that have been prepared by nick translation, end labeling, polymerization, or other labeling techniques. Specifically:

- Use mini Quick Spin DNA Columns to purify radiolabeled or fluorescent dye-labeled DNA (≥20 bp) with ≥90% recovery
- Use mini Quick Spin RNA Columns to purify radiolabeled RNA (≥20 bases) with ≥80% recovery
- Use mini Quick Spin Oligo Columns to purify radiolabeled oligonucleotides (≥8 bases) with ≥80% recovery

Assay Time

- Hands-on time: 2 min
- Total time: 7 min

2. How to use this product

2.1 Before you Begin

Precautions for Handling Radioactive Compounds

The primary application for mini Quick Spin Columns is the purification of radiolabeled nucleic acids. When purifying radiolabeled compounds with the columns, always take certain precautions, including:

- Wearing protective gloves
- Wearing safety glasses
- Working behind a lucite shield

For a thorough overview of precautions appropriate for handling radioactive compounds, see reference 1.

Calculation of Proper Centrifugation Speed

The separation procedure described in Table 2 (Section 2.2) below will not work correctly unless the columns are centrifuged at a centrifugal force (RCF) of 1,000 × *g*. Since RCF depends on the radius of the microcentrifuge rotor, as well as its speed (rpm), you will need to calculate the speed at which your microcentrifuge generates an RCF of 1,000 × *g*. Use this formula to convert microcentrifuge speed (rpm) into centrifugal force (RCF):

$$\mathbf{RCF = 1.12 \times r \times (speed/1{,}000)^2}$$

Definition of terms:

RCF = centrifugal force (× *g*)
r = radius of centrifuge rotor, measured in millimeters, from the center of the rotor to the middle of the mini Quick Spin Column
speed = rotor speed in revolutions per min (rpm)

Calculation example:

For an Eppendorf Model 5415C variable-speed microcentrifuge with an 18-position fixed-angle rotor, r = 73 mm, and the speed needed to generate an RCF of 1,000 × *g* is 3,500 rpm, as calculated below:

$$1{,}000\ g = 1.12 \times 73\ mm \times (speed/1{,}000)^2$$
$$= 81.76 \times (speed/1{,}000)^2$$
$$speed/1{,}000 = (1{,}000/81.76)^{1/2}$$
$$speed = 3{,}500\ rpm$$

Sample Material

- For DNA or RNA columns: 20 to 75 µl nucleic acid-labeling mixture
- For Oligo columns: 20 to 50 µl oligonucleotide-labeling mixture

2.2 Procedures for Purifying Labeled Nucleic Acids with Any mini Quick Spin Column

Experimental Overview

The following tables summarize the steps for preparing any mini Quick Spin Column, and purifying a nucleic acid sample with the column.

Tab. 1: Preparing the column

①	Resuspend column matrix.
②	Remove top cap, then snap off bottom tip, and place column into sterile microcentrifuge tube.
③	Spin column (1,000 × *g*, 1 min) to pack the column and remove residual buffer.

Tab. 2: Purifying the sample

①	Carefully apply sample to center of column bed.
②	Spin column (1,000 × *g*, 4 min).
③	Recover eluate containing the nucleic acids.

The total time required to complete both the procedures listed in Table 1 (preparing the column) and Table 2 (purifying the sample) is approximately 7 min.

Preparing the Column

Use the following procedure to prepare any mini Quick Spin Column.

❶ Evenly resuspend the Sephadex matrix in the column buffer by doing either of the following:

- Vigorously invert the column several times. Recommendation: Flick the column sharply several times (while it is inverted and while it is upright) to help resuspend the matrix.

or

- Gently vortex for 3 to 5 sec at low speed.

⚠ Do not vortex the column at medium or high speed, or for periods longer than 5 sec. Excessive vortexing may crush the matrix and lead to contamination of the purified sample with unincorporated nucleotides.

🔍 This step will take 5 to 15 sec.

❷ To prevent the formation of a vacuum (which can cause uneven buffer flow), remove the ends from the column in the following order:

- First, remove the top cap from the column.

⚠ If the cap is filled with Sephadex, put the cap back on the column and remix column contents (as in step 1) until most of the matrix is in the body of the column rather than in the cap.

- Then, snap off the bottom tip.

🔍 When the ends are removed, a small amount of liquid may escape the column and a small amount of Sephadex may remain in the cap. These small losses will not affect column performance.

🔍 This step will take 5 to 20 sec.

❸ Remove excess buffer and pack the column as follows:

- Place column in a sterile 1.5 ml microcentrifuge tube.
- Place the tube in a microcentrifuge rotor.

🔍 To properly attach the rotor lid, turn the microcentrifuge tube so that the flip-top cap faces the inside of the rotor. (There is a v-shaped notch in the support ring of the column to help align the column with the rotor.)

- Centrifuge at 1,000 × *g* for 1 min at +15 to +25°C.

⚠ Start the timer once the microcentrifuge has reached the recommended speed:

🔍 To calculate the microcentrifuge speed needed to generate a centrifugal force of 1,000 × *g*, see Section 2.1 within these Instructions for Use.

⚠ Do not pulse the microcentrifuge. Do not exceed the speed calculated in Section 2.1.

- Discard the collection tube with the eluted buffer.

🔍 During packing, the column matrix normally pulls away from the sides of the tube.

🔍 This step will take 1.5 min.

❹ Is the isolated nucleic acid to be used in a fluorescent sequencing reaction?
- If no, then skip this step and go to Step 5.
- If yes, then exchange the buffer in the column for water, as follows:
- Place the packed column in a 1.5 ml microcentrifuge tube.
- While keeping the column upright, apply 300 µl sterile distilled water to the center of the column bed.
- Centrifuge the tube at 1,000 × *g* for 2 min at +15 to +25°C.
- Discard the collection tube and eluted buffer.
- Go to Step 5.

The extra buffer exchange step minimizes the amount of salt in the final purified nucleic acid. Minimal salt in the final product means that, when concentrated, the sample will run cleanly in sequencing applications.

This step will take 2.5 min.

❺ Use the column immediately (as detailed in below).

⚠ Delay will cause the column matrix to dry out. A dry column will not properly perform.

Purifying the Sample

After preparing any mini Quick Spin Column according to the procedure in previous procedure, use the procedure below to purify a nucleic-acid sample with the prepared column.

❶ While keeping the column upright, do the following:
- Place the prepared column (from step 5, Preparing the Column) in a clean, sterile 1.5 ml microcentrifuge tube.
- Very slowly and carefully apply the sample to the center of the column bed. Do not apply the sample to the side of the column. Any sample on the side of the column will bypass the separation matrix and will arrive in the collection tube without being fractionated.

  Use 20 to 50 µl sample for the mini Quick Spin Oligo Column; 20 to 75 µl sample for the mini Quick Spin DNA or RNA Column. Do not overload the column.

  This step will take 5 to 15 sec.

❷ Centrifuge the tube at 1,000 × *g* for 4 min at +15 to +25°C in the microcentrifuge.

  ⚠ Do not exceed the centrifugation speed calculated in Section 2.1.

❸ Save the eluate in the second collection tube. It contains the purified nucleic acid.

  Discard the mini Quick Spin Column in an appropriate waste receptacle.

3. Results

Separation of Nucleic Acids from Unincorporated Nucleotides

In the above test, unincorporated nucleotides were separated from:

- DNA of ≥20 bp (with the mini Quick Spin DNA Column)
- RNA of ≥20 bases (with the mini Quick Spin RNA Column)
- Oligonucleotides of ≥8 bases (with the mini Quick Spin Oligo Column)

4. Troubleshooting

Problem	Possible Cause	Recommendation
Dilution of final sample	Excess packing buffer was not removed before sample application	Before applying sample, centrifuge column at 1,000 × *g* for 1 min to pack the matrix. Discard eluate. To eliminate any liquid remaining atop the column after the first spin, perform an additional 1 min spin at 1,000 × *g*.
Purified nucleic acid contaminated with unincorporated nucleotides	• Sample applied to sides of column, allowing molecules to flow around, rather than through the matrix (without purification)	• Apply sample directly to center of the column bed.
	• Column overloaded	• Do not apply more than the maximum recommended sample volume.
	• Centrifugation speed was too fast, causing column matrix to collapse, and unincorporated nucleotides to pass freely through column	• Do not centrifuge the columns faster than the recommended speed.
	• Column was vortexed too long or too vigorously during matrix resuspension	• Do not vortex the column for longer than 5 sec. • Vortex the column at low speed only. Do not use medium or high speed.
Poor recovery or no recovery of nucleic acid	• Centrifugation speed was too fast (see previous), or centrifugation time too short	• Do not centrifuge the columns faster than the recommended speed.
	• Matrix not evenly resuspended prior to packing step	• To fully resuspend the matrix before packing step, do one of the following: – Invert column vigorously several times, and flick the column sharply to help resuspend the matrix. – Vortex column gently (5 sec or less, low speed).
	• Sample volume too small (< 20 µl)	• Do one of the following: – Add 1× STE buffer to sample until the total sample volume is 20 µl. – After applying sample, add 1× STE buffer to the matrix. Total volume applied (sample + STE buffer) **MUST NOT** be greater than the maximum sample volume recommended for the column.

5. Additional Information on this Product

5.1 How this Product Works

The method uses gel-filtration chromatography, which separates molecules based upon their relative sizes. During centrifugation, mini Quick Spin Columns allow larger molecules (DNA, RNA, or oligonucleotides) to pass through quickly while retaining smaller molecules (such as unincorporated nucleotides). This rapid separation of larger from smaller molecules may be performed in a conventional tabletop microcentrifuge.

5.2 Reference

Zoon, R.A. "Safety with 32P- and 35S-labeled compounds." (1987) *Methods Enzymol.* **152**, 25–29.

5.3 Analysis

Each lot of mini Quick Spin Columns is tested to ensure:

- **Maximum retention of unincorporated nucleotides** (≥99% of nucleotides applied to the mini Quick Spin DNA or RNA Column; ≥90% retention of nucleotides applied to the mini Quick Spin Oligo Columns). Potassium ferricyanide dye (MW = 329.3) was loaded onto a Quick Spin Column and processed according to the Instructions for Use. Greater than 99% (DNA, RNA) or 95% (oligo) of the dye is retained on the column.
- **Absence of DNase contamination**. No DNA degradation products were observed in the following test: 0.5 µg of pBR322 DNA was incubated for 6 hours at +37°C in 25 µl of mini Quick Spin Column eluate containing 1 mM $MgCl_2$. After incubation, the DNA was electrophoretically analyzed in a 1% agarose gel. The gel was stained with ethidium bromide.
- **Absence of RNase activity (mini Quick Spin RNA Columns only).** No RNA-degradation products were observed in the following test: 2.4 µg of MS2 RNA was incubated for 6 hours at +37°C in 15 µl eluate from a mini Quick Spin RNA column. After incubation, 1 µg of the RNA was electrophoretically analyzed in a 1.0% agarose gel. The gel was stained with ethidium bromide.

6. Supplementory Information

6.1 Conventions

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

Text Convention	Usage
Numbered stages labeled ①, ②, *etc.*	Stages in a process that usually occur in the order listed
Numbered instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed

Symbols In this document, the following symbols are used 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.

6.2 Changes to Previous Version

- Editoral changes
- Trademarks updated

6.3 Ordering Information

Related products This table lists products for preparing labeled nucleic acids that may be purified with the mini Quick Spin Columns.

Product	Pack Size	Cat. No.	Use
Nick Translation Kit	1 kit (50 reactions)	10 976 776 001	DNA labeling
Random Primed DNA Labeling Kit	1 kit (50 reactions)	11 004 760 001	DNA labeling
SP6/T7 Transcription Kit	1 kit	10 999 644 001	RNA labeling

6.4 Trademarks

6.5 License Disclaimer

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