For life science research only.
Not for use in diagnostic procedures.

mini Quick Spin Columns

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

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

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	mini Quick Spin DNA Columns	▪ Prepared by suspending Sephadex G-50 matrix in 1x 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.	11 814 419 001	1 zippered bag, 50 columns
2	mini Quick Spin RNA Columns	▪ Prepared by suspending Sephadex G-50 matrix in 1x 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. ▪ Columns are treated with diethylpyrocarbonate (DEPC) before filling to ensure RNase-free columns.	11 814 427 001	1 zippered bag, 50 columns
3	mini Quick Spin Oligo Columns	▪ Prepared by suspending Sephadex G-25 matrix in 1x 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.	11 814 397 001	1 zippered bag, 50 columns

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	mini Quick Spin DNA Columns	Store at +2 to +8°C. ⚠ ***Do not freeze.***
2	mini Quick Spin RNA Columns	
3	mini Quick Spin Oligo Columns	

1.3. Additional Equipment and Reagent required

For column preparation and sample preparation

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

1.4. Application

The mini Quick Spin columns are designed for quick and total removal of unincorporated nucleotides, such as 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.

- 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.

1.5. Preparation Time

Assay Time

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

2. How to Use this Product

2.1. Before you Begin

Sample Materials

DNA or RNA columns

20 to 75 µl nucleic acid labeling mixture.

Oligo columns

20 to 50 µl oligonucleotide labeling mixture.

General Considerations

Calculation of centrifugation speed

The separation procedure 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):

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

- 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 minute (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 × 73 mm × (speed/1,000)^2 = 81.76 x (speed/1,000)^2
- speed/1,000 = (1,000/81.76)$^{1/2}$
- speed = 3,500 rpm

Safety Information

Precautions

The primary application for mini Quick Spin Columns is the purification of radiolabeled nucleic acids. When purifying radiolabeled compounds with the columns, always take precautions:
- Wear protective gloves.
- Wear safety glasses.
- Work behind a lucite shield.

2.2. Protocols

Experimental overview

Prepare the column:

① Resuspend column matrix.

② Remove top cap, snap off bottom tip, and place column into autoclaved microcentrifuge tube.

③ Spin column 1 minute at 1,000 × *g* to pack the column and remove residual buffer.

Purify the sample:

① Carefully apply sample to center of column bed.

② Spin column 4 minutes at 1,000 × *g*.

③ Recover eluate containing the nucleic acids.

The total time required to prepare the column and purify the sample is approximately 7 minutes.

Column preparation

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

❶ (5 to 15 seconds) Evenly resuspend the Sephadex matrix in the column buffer by doing one of two methods:
– Vigorously invert the column several times.

⚠ ***Flick the column sharply several times while it is inverted and while it is upright to help resuspend the matrix.***

– Alternatively, gently vortex for 3 to 5 seconds at low speed.

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

2. (5 to 20 seconds) To prevent the formation of a vacuum, which can cause uneven buffer flow, 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.*

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3. Remove excess buffer and pack the column as follows:

– Place column in a autoclaved 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.*

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4. (90 seconds) Centrifuge at 1,000 × *g* for 1 minute 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,* **General Considerations**.

⚠ ***Do not pulse the microcentrifuge and do not exceed the speed calculated in section, General Considerations.***

– Discard the collection tube with the eluted buffer.

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

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5. (2.5 minutes) If the isolated nucleic acid is to be used in a fluorescent sequencing reaction, exchange the buffer in the column with water.

ⓘ *If the isolated nucleic acid is not going to be used in a fluorescent sequencing reaction, proceed to Step 6.*

– Place the packed column in a 1.5 ml microcentrifuge tube.

– While keeping the column upright, apply 300 µl autoclaved double-distilled water to the center of the column bed.

– Centrifuge the tube at 1,000 × *g* for 2 minutes at +15 to +25°C.

– Discard the collection tube and eluted buffer and proceed to Step 6.

ⓘ *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.*

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6. Use the column immediately, see section, **Sample purification**.

⚠ ***Any delay will cause the column matrix to dry out. A dry column will not perform properly.***

Sample purification

After preparing any mini Quick Spin Column according to section, **Column preparation**, purify the nucleic acid sample with the prepared column:

1. (5 to 15 seconds) While keeping the column upright, place the prepared column from **Step 6, Column preparation** into a clean, autoclaved 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 Columns. Do not overload the column.*

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2. Centrifuge the tube at 1,000 × *g* for 4 minutes at +15 to +25°C.

⚠ ***Do not exceed the centrifugation speed calculated in section, General Considerations.***

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3. 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.*

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3. Results

Separation of nucleic acids from unincorporated nucleotides

- Exclusion limit of DNA: ≥20 bp
- Exclusion limit of RNA: ≥20 bases
- Exclusion limit of oligonucleotides: ≥8 bases

4. Troubleshooting

Observation	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 minute to pack the matrix. Discard eluate. *To eliminate any liquid remaining atop the column after the first spin, perform an additional 1 minute 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 is 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 seconds.
		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 seconds or less at low speed.
	Sample volume too small (<20 µl).	Do one of the following: ▪ Add 1x STE buffer to sample until the total sample volume is 20 µl. ▪ After applying sample, add 1x 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. Test Principle

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, such as 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. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Trademarks

All product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

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

1121.05078385001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany