

mini Quick Spin Columns

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

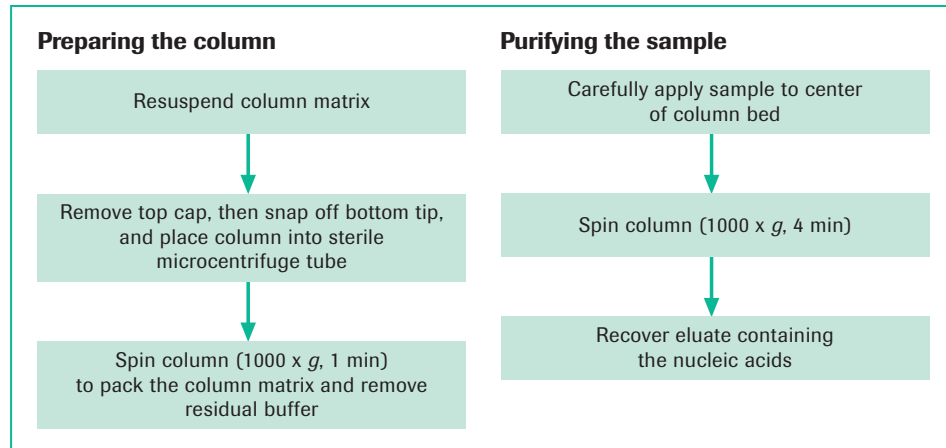
Principle	The method uses gel filtration chromatography, which separates molecules based upon their relative size. During centrifugation, mini Quick Spin Columns allow larger molecules (DNA, RNA, or oligonucleotides) to pass through quickly while retaining smaller molecules (such as unincorporated nucleotides). The rapid separation of larger from smaller molecules may be performed in a conventional tabletop microcentrifuge.
Starting material	<ul style="list-style-type: none"> ● For 'DNA or RNA' columns: 20 – 75 µl nucleic acid labeling mixture ● For 'Oligo' columns: 20 – 50 µl oligonucleotide labeling mixture
Application	<p>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:</p> <ul style="list-style-type: none"> ● 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
Time required	<ul style="list-style-type: none"> ● Hands-on time: 2 min ● Total time: 7 min (additional 1 min if buffer exchange is needed)
Results	<p>Removal of unincorporated nucleotides</p> <ul style="list-style-type: none"> ● mini Quick Spin DNA or RNA Column ≥ 99 % retention ● mini Quick Spin Oligo Column ≥ 90 % retention <p>Recovery of labeled probes</p> <ul style="list-style-type: none"> ● mini Quick Spin DNA Columns ≥ 90 % ● mini Quick Spin RNA/Oligo Columns ≥ 80 % <p>Exclusion limit</p> <ul style="list-style-type: none"> ● mini Quick Spin DNA/RNA Columns ≥ 20 bp ● mini Quick Spin Oligo Columns ≥ 8 bases
Benefits	<ul style="list-style-type: none"> ● Saves time and effort, because columns are ready to use with any standard microcentrifuge ● Provides more nucleic acid in less time, because two step procedure mean minimal sample loss and increased yield ● Gives reproducible results, because columns are quality tested to ensure high recovery of labeled DNA, RNA and oligonucleotides with a maximum retention of unincorporated nucleotides



How to use the columns

I. Flow diagram

The following flow diagram summarizes the steps for preparing any mini Quick Spin Column and purifying a nucleic acid sample with the column.



II. Additional materials needed

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

III. Preparing the column

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

- 1 Evenly resuspend the Sephadex matrix in the column buffer by doing either of the following:
 - ▶ Invert the column vigorously several times.
 - or
 - ▶ Vortex gently for 3 – 5 s at low speed.

! Do not vortex the column at medium or high speed or for periods longer than 5 s. Excessive vortexing may crush the matrix and lead to contamination on the purified sample with unincorporated nucleotides.
- 2 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.



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- 3 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 attach the rotor lid properly, 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 1000 x *g* for 1 min at 15 to 25°C.
 - ▶ 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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- 4 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 double distilled water to the center of the column bed.
 - ▶ Centrifuge the tube at 1000 x *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.*
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- 5 Use the column immediately (as detailed below).
 - ! *Delay will allow the column matrix to dry out. A dry column will not perform properly.*
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IV. Purifying the sample

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

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- 1 While keeping the column upright, do the following:
 - ▶ Place the prepared 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 – 50 µl sample for the mini Quick Spin Oligo Column; 20 – 75 µl sample for the mini Quick Spin DNA or RNA Column. Do not overload the column.*
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- 2 Centrifuge the tube at 1000 x *g* for 4 min at 15 to 25°C in the microcentrifuge.
 - 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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V. Troubleshooting the mini Quick Spin protocol

This table describes various problems that may occur during the purification procedures and recommendations for avoiding them.

If you get...	Then, the cause may be...	And you should...
Dilution of final sample	Excess packing buffer wasn't removed before sample application	<ul style="list-style-type: none"> ▶ Before applying sample, centrifuge column at 1000 x g for 1 min to pack the matrix. Discard eluate. ! <i>To eliminate any liquid remaining atop the column after the first spin, perform an additional 1 min spin at 1000 x g.</i>
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)	<ul style="list-style-type: none"> ▶ Apply sample directly to center of the column bed.
	Column overloaded	<ul style="list-style-type: none"> ▶ Do not apply more than the maximum recommended sample volume.
	Centrifugation speed too fast, causing column matrix to collapse and unincorporated nucleotides to pass freely through column	<ul style="list-style-type: none"> ▶ Do not centrifuge the columns faster than the recommended speed.
	Column was vortexed too long or too vigorously during matrix resuspension	<ul style="list-style-type: none"> ▶ Do not vortex the column for longer than 5 s. ▶ Vortex the column at low speed only. Do not use medium or high speed.
Poor recovery or no recovery of nucleic acid	Centrifugation speed too fast (see above) or centrifugation time too short	<ul style="list-style-type: none"> ▶ Do not centrifuge the columns faster than the recommended speed.
	Matrix not evenly resuspended prior to packing step	<ul style="list-style-type: none"> ▶ To fully resuspend the matrix before packing step, do one of the following: <ul style="list-style-type: none"> ▶ Invert column vigorously several times and flick the column sharply to help resuspend the matrix. ▶ Vortex column gently (5 s or less, low speed).
	Sample volume too small (<20 µl)	<ul style="list-style-type: none"> ▶ Do one of the following: <ul style="list-style-type: none"> ▶ Add 1x STE buffer to sample until the total sample volume is 20 µl. ▶ After applying sample, add 1x STE buffer to the matrix. ! <i>Total volume applied (sample + STE buffer) MUST NOT be greater than the maximum sample volume recommended for the column.</i>



Typical result with the columns

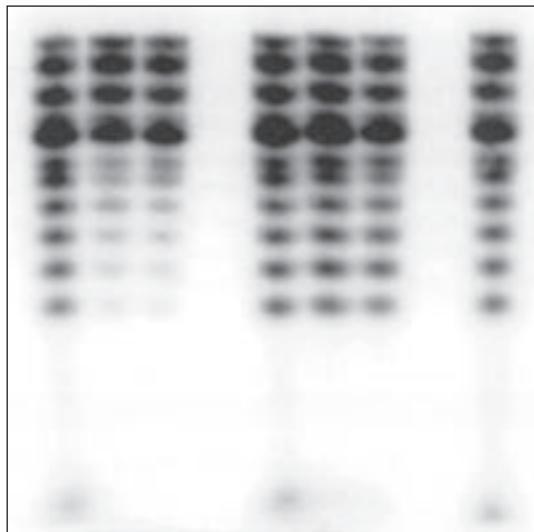


Figure 53: Removal of Unincorporated Radiolabeled Nucleotides from 5' End-labeled Oligomers using the Mini Quick Spin DNA and Oligo Columns. Various oligomers, ranging in size from 32 bp to 8 bp, were end-labeled using the 5' End-Labeling Kit and $\gamma^{32}\text{P}$ -ATP and purified using either the mini Quick Spin DNA or Oligo Columns to remove unincorporated nucleotide, per manufacturer instructions. One fifth of the isolated sample was electrophoresed through a 20% acrylamide gel in 1x TBE for 5 – 6 hours at 150 V. The gel was overlaid with plastic wrap and exposed to Lumi-Film for approximately 18 h.

Lane 1: Oligo Marker Control

Lane 2: Oligo Marker/miniQuick Spin DNA Column

Lane 3: Oligo Marker/mini Quick Spin DNA Column

Lane 4: –

Lane 5: Oligo Marker Control

Lane 6: Oligo Marker/mini Quick Spin Oligo Column

Lane 7: Oligo Marker/mini Quick Spin Oligo Column

Lane 8: –

Lane 9: Oligo Marker Control

Result: Removal of Unincorporated Radio-labeled Nucleotides from 5' End-labeled Oligomers using the Mini Quick Spin DNA and Mini Quick Spin Oligo Columns

References

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