

Quick Spin Columns

G-25 or G-50 Sephadex Columns for Radiolabeled DNA Purification

G-25 or G-50 Sephadex Columns for Radiolabeled RNA Purification

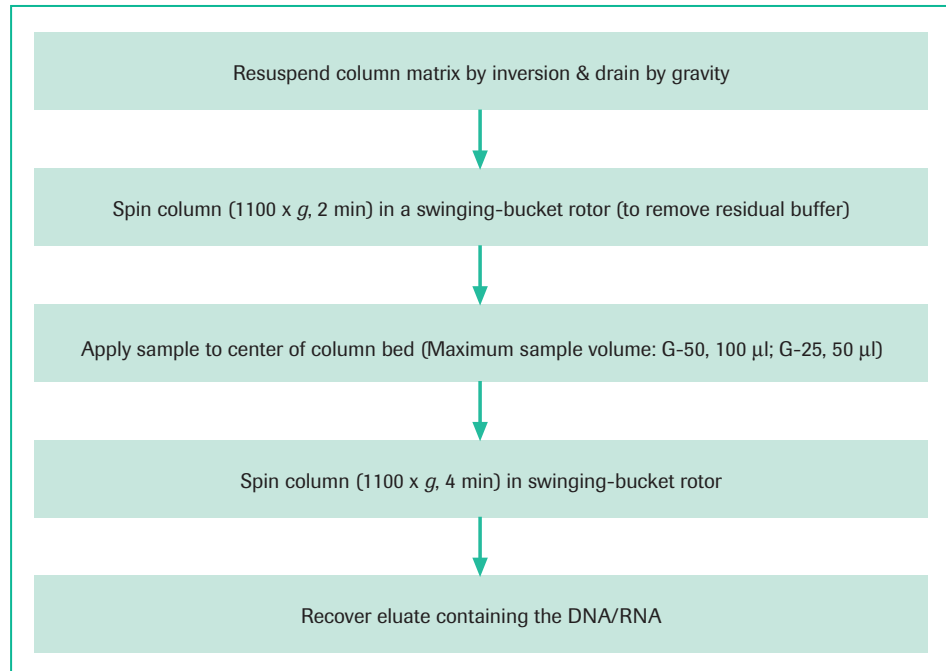
Cat. No. 11 273 922 001	20 columns for DNA purification (Sephadex G-25)
Cat. No. 11 273 949 001	50 columns for DNA purification (Sephadex G-25)
Cat. No. 11 273 965 001	20 columns for DNA purification (Sephadex G-50)
Cat. No. 11 273 973 001	50 columns for DNA purification (Sephadex G-50)
Cat. No. 11 273 990 001	20 columns for RNA purification (Sephadex G-25)
Cat. No. 11 274 015 001	20 columns for RNA purification (Sephadex G-50)

Principle	Quick Spin columns contain gel filtration matrices which allow large molecules (<i>e.g.</i> , DNA or RNA) to pass through quickly while retaining small molecules (<i>e.g.</i> , nucleotides). The Quick Spin format improves the molecular sieving concept by using centrifugation to separate DNA or RNA rapidly and cleanly from small contaminants.	
Starting material	<ul style="list-style-type: none"> ● Nucleic acids from labeling reactions 	
Application	<ul style="list-style-type: none"> ● Quick and efficient removal of non-incorporated precursors from: <ul style="list-style-type: none"> ● Labeled DNA (from nick translation, end-labeling, polymerization, and other labeling reactions) ● Labeled RNA (from end-labeling or polymerization reactions) 	
Time required	<ul style="list-style-type: none"> ● Total time: 8 min ● Hands-on time: 2 min 	
Results	<p>G-50 Sephadex Columns</p> <ul style="list-style-type: none"> ● Exclusion limit: DNA: 72 bp / RNA: 72 bp ● Recovery: DNA: $\geq 90\%$ / RNA: $\geq 80\%$ ● Retention of nucleotides: DNA: $>99\%$ / RNA: $>99\%$ ● Sample volume: DNA: up to 100 μl / RNA: up to 100 μl <p>G-25 Sephadex Columns</p> <ul style="list-style-type: none"> ● Exclusion limit: DNA: 10 – 12 bp / RNA: 10 – 12 bp ● Recovery: DNA: $\geq 80\%$ / RNA: $\geq 80\%$ ● Retention of nucleotides: DNA: $\geq 95\%$ / RNA: $\geq 95\%$ ● Sample volume: DNA: up to 50 μl / RNA: up to 50 μl 	
Benefits	<ul style="list-style-type: none"> ● Saves time and effort, because columns are ready-to-use ● Provides more nucleic acids in less time, because fewer steps mean minimal sample loss and increased yield ● Gives reproducible results, because columns are quality tested to ensure: <ul style="list-style-type: none"> ● High recovery of radiolabeled DNA, RNA, and poly(A) ● Maximum retention of unincorporated nucleotides ● Absence of DNases in DNA columns ● Absences of DNases and RNases in RNA columns 	

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How to use the columns

I. Flow diagram



II. Package contents

- Each column contains 0.8 ml bed volume of pre-swollen Sephadex G-25 (fine) or G-50 (fine) in STE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, 100 mM NaCl).
- Each column unit contains a ready-to-use column plus two collection tubes (one for draining the column buffer and one for collecting the purified nucleic acid sample).



The Quick Spin columns for radiolabeled RNA purification are packed in ziplock bags to prevent contamination.

III. Additional materials needed

- Tabletop or low-speed floor model centrifuge
- Swinging-bucket rotor



IV. Protocol for purifying radiolabeled nucleic acids


- 1 Remove the column from the storage bag, and gently invert it several times to resuspend the matrix.
- 2 Remove most of the buffer from the column as follows:
 - ▶ Remove the top cap from the column, then remove the bottom tip.
 - ▶ Drain the column by gravity, then discard the eluate.
- 3 Remove residual buffer from the column as follows:
 - ▶ Place the column in a collection tube.
 - ▶ Centrifuge at 1100 x *g* for 2 min in a swinging-bucket rotor.
 - ▶ Discard the collection tube with the eluted buffer.
- 4 While keeping the column upright, very carefully apply the sample to the center of the column bed.
 - ! Apply 20 – 50 μ l sample to a G-25 column; 20 – 100 μ l to a G-50 column.
- 5 While keeping the column upright, place the column in the second collection tube.
- 6 Centrifuge the tube at 1100 x *g* for 4 min in a swinging-bucket rotor.
- 7 Save the eluate from the second collection tube. It contains the purified sample.
- 8 Discard the column in a radioactive waste container.

V. Troubleshooting the Quick Spin protocol

If you get...	Then, the cause may be...	And you should...
Poor sample recovery	Tipping of the column, which causes backflow of sample and reduced nucleic acids recovery	<ul style="list-style-type: none"> ▶ Keep the column upright during and after application of sample, especially after centrifugation.
	Wrong g-force used during centrifugation	<ul style="list-style-type: none"> ▶ Use 1100 x <i>g</i> for centrifugation spins. ▶ Be sure the centrifuge is correctly calibrated.
	Wrong rotor	<ul style="list-style-type: none"> ▶ Use swinging-bucket rotor. ! A fixed-angle rotor will cause lower yields.
	Too much or too little DNA/RNA loaded on column	<ul style="list-style-type: none"> ▶ Apply a sample containing 0.02 – 1.0 mg/ml nucleic acid. At >1.0 mg/ml, the sample is viscous and may not migrate through the columns easily, leading to poor recovery and/or contamination with smaller molecules. At <0.02 mg/ml, DNA/RNA recovery may be low. ▶ To improve recovery of dilute samples, add carrier (glycogen, tRNA or sperm DNA) to the sample.



V. Troubleshooting the Quick Spin protocol, continued

If you get...	Then, the cause may be...	And you should...
Nucleotide contamination of final sample	Sample applied to side of the column matrix, allowing nucleotides to flow around the matrix	▶ Carefully apply the sample to the center of the column matrix.
	Column overloaded, which causes nucleotides to flow through the matrix	▶ Apply a sample containing 0.02 – 1.0 mg/ml nucleic acid. ▶ Apply no more than 50 µl sample to a G-25 column; no more than 100 µl to a G-50 column.
	Wrong g-force during centrifugation	▶ Use 1100 x g for centrifugation spins. ▶ Be sure the centrifuge is correctly calibrated.
	Wrong rotor	▶ Use swinging-bucket rotor.  <i>A fixed-angle rotor may cause nucleotide contamination of final sample.</i>

Reference

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