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

Quick Spin Columns for radiolabeled DNA purification

Sephadex G-25

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G-25 Sephadex Columns for radiolabeled DNA purification (Exclusion limit: <10 base pairs).

Cat. No. 11 273 922 001 20 columns
Cat. No. 11 273 949 001 50 columns

Store product at +2 to +8°C.

1. General Information

1.1. Contents

Package	Label	Function / Description	Catalog number	Content
1	Quick Spin Columns for radiolabeled DNA purification Sephadex G-25 (Fine)	▪ Ready-to-use, pre-packed columns. ▪ Each column contains a 0.8 ml bed volume of pre-swollen Sephadex G-25. ▪ Suspension of Sephadex G-25 in STE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 100 mM NaCl). ▪ Each column unit consists of a ready-to-use column plus two collection tubes, one for draining the column buffer and one for collecting the purified DNA sample. *The column units are packaged in heat-sealed storage containers to prevent contamination.*	11 273 922 001	20 columns plus 40 collection tubes
			11 273 949 001	50 columns plus 100 collection tubes

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the columns are stable through the expiry date printed on the label.
To avoid contamination of the columns and collection tubes, leave them in the storage bag until just before use.

Package	Label	Storage
1	Quick Spin Columns for radiolabeled DNA purification Sephadex G25 (fine)	Store at +2 to +8°C. ***Do not freeze.***

1.3. Additional Equipment and Reagent required

For purification of radiolabeled DNA

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

1.4. Application

Ready-to-use disposable column units designed to quickly and efficiently remove unincorporated precursors from DNA labeled by:

- Nick translation
- End labeling
- Polymerization reactions
- Other labeling techniques

Quick Spin Columns are designed for use in low-speed, swinging-bucket centrifuges.

2. How to Use this Product

2.1. Before you Begin

General Considerations

General handling recommendations

For instructions on using Quick Spin Columns to purify radiolabeled DNA, see section, **Protocols**.

Precautions

As with all procedures that use radioactive material, take appropriate precautions when working with any type of hazardous materials.

- Wear protective gloves and safety glasses, and use Lucite shielding.
- Use a double-containment system, for example, place the Quick Spin Column/collection tube in a conical plastic carrier tube whenever you work with radioactive reagents or other hazardous materials.
- Verify that the filter is properly seated in the column, present but not tilted so as to allow Sephadex to pass through.

⚠ ***Read the section, Protocols and Notes on centrifugation before proceeding.***

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. For example, with an IEC HN-SII centrifuge, 1,100 × *g* corresponds to 3,000 rpm. Make sure that the centrifuge is accurately calibrated. Information from the centrifuge manufacturer will allow you to convert rpm to *g*-force.
- Quick Spin Columns can be centrifuged in most tabletop/clinical centrifuges and low-speed floor model centrifuges that use swinging-bucket rotors.

  ⚠ ***Use a swinging-bucket rotor instead of a fixed-angle rotor.***

  ℹ *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 DNA sample is likely to slide down the sides of the tube instead of flowing through the medium. This results in poor retention of nucleotides and decreased recovery of DNA.*
- If adapter/carrier tubes are used, it may be necessary to use oversized forceps to remove the column from the tube.

2.2. Protocols

Purification of radiolabeled DNA

1. Remove the column from the storage container and gently invert it several times to resuspend the medium.

2. Remove the top cap from the column, then remove the bottom tip.

   ℹ *This sequence is necessary to avoid creating a vacuum and uneven flow of the buffer.*

   – Allow the buffer to drain by gravity and discard.

3. Place the column in a collection tube and centrifuge at 1,100 × *g* for 2 minutes.

   – Discard the collection tube and the eluted buffer.

4. Keeping the column in an upright position, very carefully apply the DNA sample (up to 50 µl) to the center of the column bed.

   ℹ *Avoid applying the sample to the sides of the column, as this may cause uneven sample absorption and incomplete desalting.*

   ℹ *Overloading the column (volume >50 µl) also results in nucleotides flowing through, contaminating the DNA sample.*

   ⚠ ***If utilizing the optional wash step (Step 7), sample load should not exceed 30 µl.***

5 Being careful to keep the column in an upright position, place the column in the second 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 recovery.***

6 Centrifuge for 4 minutes at 1,100 × *g*.
– Save the eluate from the second collection tube.
This contains your purified DNA sample.

7 **Optional wash step**: Remove column from centrifuge and carefully load up to 30 µl of autoclaved water onto the center of the column bed.
– Centrifuge for 4 minutes at 1,100 × *g*.
The optional wash step is required for maximal recovery of radiolabeled DNA (≥80%). If utilizing the optional wash step, the initial sample load (Step 4) should not exceed 30 µl, otherwise retention of unincorporated nucleotides may fall below 95%. Maximal retention of unincorporated nucleotides (≥99%) is achieved by omitting the wash step, but may result in reduced radiolabeled DNA recovery.

8 Save the combined eluate from the second collection tube.
This contains your purified DNA sample.

9 Discard the column into a designated radioactive waste container.

3. Additional Information on this Product

3.1. Test Principle

Quick Spin Columns are produced with material that is shown to have a high recovery of radiolabeled DNA (≥80%). All the tedious, time-consuming steps involved in preparing columns have already been performed. The columns are pre-packed and quality-tested to ensure

- maximal retention of unincorporated nucleotides (≥95%), and
- absence of DNase contamination.

3.2. Quality Control

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

4. Supplementary Information

4.1. 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.

4.2. Changes to previous version

Layout changes.

Editorial changes.

4.3. Trademarks

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

4.4. License Disclaimer

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

4.5. Regulatory Disclaimer

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

4.6. Safety Data Sheet

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

4.7. 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.

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