



Enzymax LLC, 870 Corporate Dr., Suite 201; Web: [www.enzymax.net](http://www.enzymax.net); Tel: 859-219-8482; Fax: 859-219-0653

Cat. #	Description	Quantity	Price
G-25	<a href="#">Sephadex G-25 spin column, dry resin</a>	30	\$79
G-25W	<a href="#">Sephadex G-25 spin column, wet resin</a>	30	\$89
ESC7	Empty spin column without resin	50	\$49
G-25W-SC	Sephadex G-25 in screw cap spin column, pre packed wet resin	30	\$109
G-25D-SC	Sephadex G-25 in screw cap spin column, pre packed dry resin	30	\$109
G-50W-SC	Sephadex G-25 in screw cap spin column, pre packed wet resin	30	\$109
EZC116	Empty screw cap spin column with snap-off tip	50	\$79

### **Description:**

G-25 Spin Column provides a fast and efficient purification of large molecules (nucleic acids, complex carbohydrates, peptides, proteins) from small molecules (nucleotides, labels and salts). Each column contains a special frit with either pre packaged dry resin or self-loading wet resin designed to achieve high recovery (>70%) of DNA fragments (>10 bases) while removing >98% of salt, traces of phenol, probes, and dNTP's.

### **Applications:**

- Idea for the purification of oligonucleotides or very small DNA fragments (>10 bases).
- Fast & efficient removal of free and labeled dNTPs in end-labeling, nick translation, and other synthesis or labeling reactions.
- Removal of dye deoxyterminators in manual or automated sequencing reactions.
- Buffer exchange, purification of peptides or proteins.
- Commonly used for purification of protein conjugates in fluorescence, cross-linking, or other labeling reactions.

### **Protocol for G25 (dry resin) and G-25D-SC:**

**Note:** Maximum yield and efficiency are obtained with horizontal or swinging-bucket type rotors. However, microcentrifuges with fixed-angle-rotor provided acceptable performance. Do not use pulse button on a variable speed microcentrifuge, which overrides the speed setting to maximum speed.

1. Gently tap the column or spin at 1000x g for 1-2 seconds to insure the dry resin settling at the bottom of the spin column.
2. Open the top and hydrate the dry resin with 0.75 ml of RNase-free water (0.65 ml for G-25D-SC, screw cap spin column) or the buffer of your choice (typically the same buffer used in the samples).
3. **Note:** It is important to fully hydrate the dry resin before use. Swelling time: **24h** at 25°C. Reconstituted columns can be store at 4°C for several days. Longer storage can be accomplished in 10mM Sodium Azide. Allow refrigerated columns to warm to room temperature before use.
4. After the resin was completely hydrated, tap the bottom of the column to remove the air bubbles if needed. Make sure the gel is free of bubbles. Open the cap and spin the column for 2 minutes at 750x g to remove excess interstitial fluid. If there is a drop at the end of the column, blot it dry.
  - Note 1:** If using a fixed-angle microcentrifuge, keep track of the orientation of the column in the rotor. The highest point of the gel media in the column should always point toward the outside of the rotor.
  - Note 2:** Make sure columns do not crack or dry after spin, if so, resuspend the resin again and centrifuge for less time or up to 20% slower speed. Columns do not operate efficiently if they have dried out.
5. Immediately apply 20-50 µl of sample to the center of the gel bed at the top of the column carefully without disturbing the gel surface.
  - Note 1:** To obtain highest purification efficiency, do not contact the sides of the column with sample pipette tip or reaction mixture. **Note 2:** Sample volume should not exceed 100 µl.
6. Place the column with a new sample collection tube (2.0 ml) into the rotor.
  - Note:** It is important to maintain proper column orientation. The highest point of the gel media in the column should always point toward the outside of the rotor.
7. Spin the column with collection tube at 4°C for 2 minutes at 750x g and your sample will be the flow-through in the collection tube. Discard the spin column and continue with your procedure.