SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

SigmaPrep™ spin column

Catalog Number **SC1000**
Store at room temperature

TECHNICAL BULLETIN

Product Description

SigmaPrep Spin Columns are designed for fast and convenient purification of a protein or protein complex using affinity media. Immunoprecipitation or affinity purification methods are a common way to perform small-scale purification of target molecules. This product provides the tools to perform these procedures without significant loss of the affinity medium. The kit includes spin columns, collection tubes, and end caps. The column comes assembled with a 7-20 micron polyethylene frit. Each spin column fits securely in the 2 ml collection tubes. The assembly can be used in a microcentrifuge. This product provides 25 columns, allowing for collection of the unbound, wash, and elution fractions of up to 25 samples simultaneously. The researcher needs only to supply the affinity medium of choice. SigmaPrep Spin Columns have a maximum capacity of 800 µl. The addition of the end caps with this product provides a convenient storage method for the used column and can also be used for incubation without loss of sample.

Components

- 25 Spin Columns, Catalog Number HP6787
- 25 End Caps for SigmaPrep Columns, Catalog Number V2014
- 100 Collection Tubes, 2 mL, Catalog Number T5449

Equipment Required but Not Provided

- Microcentrifuge or vacuum manifold

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

Users must determine the purification procedure suitable for their applications. Many methods are available in the literature. The method selected will depend upon the sample size and the amount of affinity medium. The following procedure is provided only as a guideline.

1. Add affinity medium to the SigmaPrep Spin Column. Not more than 400 µL is suggested for optimum performance.

2. Place the spin column in a collection tube, close the lid, and place the assembly in a microcentrifuge.

3. Centrifuge at approximately 8200 × *g* (10,000 rpm in an Eppendorf® 5415C microcentrifuge) for 1 minute to remove storage buffer from the affinity medium (see Technical Tip 2).

4. Remove the spin column from the collection tube, empty contents of the tube, and return the spin column to the collection tube.

5. Add up to 600 µL of equilibration buffer (maximum volume of the collection tube) to the spin column. Close the lid.

6. Repeat steps 3 & 4 as needed.

7. Place the spin column in a new collection tube.

8. Load the sample solution onto the column. Close the lid. If incubation is desired, place end cap onto the end of the column and invert or vortex to mix. Incubate for 5 to 60 minutes at the desired temperature. Remove end cap before centrifuging.

9. Centrifuge as in step 3.

10. Remove the spin column from the collection tube. Save the eluate for later analysis, if desired.

11. Place the spin column in a new collection tube.

12. Add wash buffer to the spin column and centrifuge as in step 3. Repeat as necessary.

13. Elute the target sample into a new collection tube with elution buffer, maximum 600 µL, and centrifuge as in step 3.

14. Eluted samples can be analyzed by assay procedures such as Bradford Reagent, BCA Reagent, QuantiPro BCA Reagent, SDS-PAGE, or Western blotting.

Technical Tips

1. SigmaPrep Spin Columns can also be used with a vacuum manifold.

2. Adjust centrifuge speed for optimal flow rate, which is dependent upon the volume of resin and or sample.

3. The binding step can be done in batch format using a separate tube. Then transfer the resin to a SigmaPrep Spin Column to wash and elute the sample.

4. Bound protein can be eluted from the spin column with electrophoresis sample loading buffer for convenient loading onto SDS-PAGE gels. Add sample buffer to the affinity medium after the wash step (step 11), boil samples in the spin column, then centrifuge to collect the protein in sample buffer.

5. Samples can also be loaded on the columns, washed, and eluted by gravity flow through the medium. This technique requires an initial centrifugation step to fully wet the frit material. Purification can be carried out according to the procedure above with only the small amounts of remaining material in the end of the column and in the media needing to be removed by centrifugation.

GS,SW,PHC 07/17-1