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Product Information

SigmaPrep™ Spin Columns with Break-Away Tip

Catalog Number **MC1000**
Store at room temperature

TECHNICAL BULLETIN

Product Description
SigmaPrep Spin Columns with Break-Away Tip 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 screw caps. The column comes assembled with a 7-20 micron polyethylene frit. The researcher needs only to supply the affinity medium of choice. A maximum volume of 700 µl can be placed in each column. The column's unique design includes a break-away tip that also functions as an end plug, allowing for a convenient storage method for the used column or a method for incubation without loss of sample.

Components
- 25 Spin Columns with Break-Away Tip, Catalog Number H6912.
  Includes 25 red polypropylene screw caps
- 50 Collection Tubes, 2 mL, Catalog Number T7813 or T5449

Equipment Required
- Microcentrifuge

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. A volume of no more than 400 µl is suggested for optimum performance. Fasten the cap.
2. Break off the end tip of the spin column and set aside.
3. Place the spin column in a collection tube and place the assembly in a microcentrifuge.
4. Centrifuge at approximately 82 x *g* for 1 minute to remove storage buffer from affinity medium (see Technical Tip 2).
5. Remove the spin column from the collection tube, empty the contents of the tube, and place the spin column back in the collection tube.
6. Add up to 500 µl of equilibration buffer (maximum volume of collection tube) to the spin column. Fasten cap.
7. Repeat steps 4 & 5 as needed.
8. Load the sample solution onto the column. If incubation is desired, seal the column with screw cap and plug and invert or vortex to mix. Incubate for 5 to 60 minutes at the desired temperature. Remove cap and plug before centrifuging.
9. Centrifuge as in step 4.
10. Remove the spin column from the collection tube. Save the eluate for later analysis, if desired.
11. Add wash buffer to the spin column and centrifuge as in step 4. Repeat as necessary.

12. Elute the target sample into a new collection tube with elution buffer, maximum 500 µl, and centrifuge as in step 4.

13. Eluted samples can be analysed by assays such as Bradford Reagent, BCA Reagent, QuantiPro BCA Reagent, SDS-PAGE, or Western blotting.

Technical Tips

1. The collection tubes supplied with this product are not required for use with these columns. SigmaPrep Spin Columns with Break-Away Tip can also be used with other microcentrifuge tubes. For very low elution volumes a 1.5 ml microcentrifuge tube is recommended for easier sample collection.

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. Sample solutions 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 medium needing to be removed by centrifugation.

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