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CALBIOSORB™ Adsorbent

CALBIOSORB™ Adsorbent (50 ml)
Cat. No. 206550

Solubilization of membranes by detergents is essential for their characterization and reconstitution. However, subsequent removal of detergents, particularly the non-ionic detergents with low CMC values, is difficult to achieve.^{1,2} Dialysis is the most common method of detergent removal; however, it is ineffective for detergents of low CMC values. Dialysis can be used for detergents with high CMC values, but usually requires about 200-fold excess of detergent-free buffer with three to four changes over several days. In addition, prolonged exposure to detergents during dialysis can damage certain membrane proteins. Gel filtration, another common method for detergent removal, is highly effective in the reconstitution of AChR3, (Ca²⁺ + Mg²⁺)-ATPase,⁴ and lactose transporters.⁵ However, it gives a broader size distribution of vesicles compared to the dialysis method.⁶ Therefore, an

**CALBIOSORB™ Adsorbent,
Prepacked Columns (3 x 5 ml resin-bed columns)**
Cat. No. 206552

expeditious alternative in reconstititional studies is the removal of detergents using a resin capable of effectively binding nondialyzable detergents of low CMC. CALBIOCHEM® offers an excellent detergent removal product, CALBIOSORB™ Adsorbent. CALBIOSORB™ Adsorbent is a hydrophobic resin that is designed to eliminate unbound organic contaminants, salts, and heavy metal ions and is especially prepared for the removal of detergents from aqueous media. It is supplied in 100 mM sodium phosphate buffer, pH 7.0, containing 0.1% sodium azide and can be easily re-equilibrated with any other buffer prior to use.

The following table highlights the adsorptive capacity of CALBIOSORB™ Adsorbent as tested for a variety of commonly-used detergents.

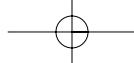
Detergent	Cat. No.	M.W.	Type	Adsorption Capacity (mg detergent/ml resin)
Cetyltrimethylammonium Bromide (CTAB)	219375	364.5	Cationic	120
CHAPS	220201	614.9	Zwitterionic	110
Cholic Acid, Sodium Salt	229101	430.6	Anionic	73
<i>n</i> -Dodecyl-β-D-maltoside, ULTROL® Grade	324355	510.6	Non-ionic	66
<i>n</i> -Hexyl-β-D-glucopyranoside	376965	264.3	Non-ionic	78
Lauryldimethylamine Oxide (LDAO)	428011	229.4	Zwitterionic	66
<i>n</i> -Octyl-β-D-glucopyranoside, ULTROL® Grade	494460	292.4	Non-ionic	132
Sodium Dodecyl Sulfate (SDS)	428015	288.5	Anionic	94
<i>n</i> -Tetradecyl-β-D-maltoside	583802	538.6	Non-ionic	161
TRITON® X-100, PROTEIN GRADE® Detergent	648463	647.0 (avg.)	Non-ionic	157
TWEEN® 20, PROTEIN GRADE® Detergent	655206	1228.0 (avg.)	Non-ionic	122

TRITON® is a registered trademark of Rohm and Haas; TWEEN® is a registered trademark of ICI.

Note: Detergent adsorption capacities were measured by allowing 1.0 g of buffer-free CALBIOSORB™ Adsorbent to equilibrate at room temperature with an excess of detergent (10.0 ml of 2.0% detergent in H₂O) for 24 hours, then measuring the amount of unadsorbed detergent remaining in the supernatant by gravimetric analysis.

References:

- Jones, O., et al. 1987. in *Biological Membranes: A Practical Approach*. (Findlay, J., and Evan, W., eds.), pp. 139-177, IRL Press, Oxford.
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- Murkejee, P. 1967. *Adv. Colloid. Interface Sci.* **1**, 241.
- Andersen, J.P., et al. 1983. *Eur. J. Biochem.* **134**, 205.
- Furth, A.J. 1980. *Anal. Biochem.* **109**, 207.
- Popat, S.L., and Changeux, J.P. 1984. *Physiol. Rev.* **64**, 1162.



Protocol for Applications Using CALBIOSORB™ Adsorbent, Prepacked Columns

1. Equilibrate the column with 4 to 5 column volumes of the sample buffer (e.g., 20 mM phosphate buffer) to remove sodium azide.
2. Apply the detergent-protein sample to the column.
3. Protein elution from the column may require several column volumes of buffer and can be monitored by UV absorption.

Protocol for Batch Applications Using CALBIOSORB™ Adsorbent

1. Wash CALBIOSORB™ Adsorbent to remove any sodium azide.
2. Calculate the amount of detergent to be removed. For example, 10 ml of 4 mM CHAPS solution contains 24.6 mg of CHAPS.
3. The amount of CALBIOSORB™ Adsorbent required for detergent removal can be determined by inserting the detergent-specific adsorption capacity from Table in the following equation:

$$\text{Amount of CALBIOSORB™ Adsorbent} = \frac{\text{Amount of detergent (mg)}}{\text{Adsorption Capacity (mg/ml)}}$$

(i.e., 24.6 mg CHAPS requires about 0.22 ml of CALBIOSORB™ Adsorbent slurry)

4. Add CALBIOSORB™ Adsorbent directly to the detergent-protein solution. Incubate for 5 minutes at room temperature or for 45 minutes on ice with occasional gentle agitation.
5. Allow the resin to settle. Decant the detergent-free supernatant containing the protein.
6. Dialysis: CALBIOSORB™ Adsorbent may be added directly to a dialysis buffer to facilitate the removal of detergents with low CMC values and to decrease the time required for dialysis when using detergents with higher CMC values. This method is advantageous in that it prevents the adsorption of proteins by the resin.

A wide variety of application-specific pH and buffer compositions (e.g., HEPES, MOPS, PIPES, Tris, etc.) may be used.

Regeneration and Storage

Regeneration: Wash with methanol followed by exhaustive washing with water. Re-equilibrate with the desired buffer used in the experiment. (NOTE: Exhaustive washing is essential to remove methanol from the resin). CALBIOSORB™ Adsorbent columns can be used up to ten times before disposal. Regeneration of prepacked CALBIOSORB™ Adsorbent columns is not recommended.

Storage: Wash the resin with a buffer containing 0.1% sodium azide and refrigerate at +4°C.

When using either the batch or column method, lower ionic strength buffers may decrease the amount of protein adsorption by the resin.

Please call our Technical Service Department or your local sales office for more information on these products.

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