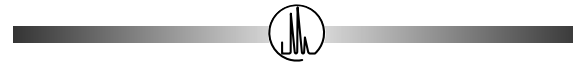

Bio-Beads® SM Hydrophobic and Polar Interaction Adsorbents

Instruction Manual

BIO-RAD

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LIT195 Rev B

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Section 1
Introduction

Bio-Beads SM adsorbents, for adsorbing organics from aqueous solutions, are neutral, macroporous polymeric Analytical Grade adsorbents of high surface area, which are intended for laboratory use only. The adsorbent is composed of a large number of highly crosslinked microspheres. This macroreticular structure gives it high surface area and uniform pore sizes. Bio-Beads adsorbents can be used with a variety of solvents, including alcohols, petroleum ether, diethyl ether, hexane, and solvent mixtures, as well as with aqueous media. They have excellent physical stability and will withstand temperatures up to 250 °C and pressures to 1,200 psi. Biotechnology Grade Bio-Beads SM adsorbents are specifically certified to contain less than 100 microorganisms per gram.

All Bio-Beads SM adsorbents are available in bulk packages.

Table 1. Description of Bio-Beads SM Adsorbents

	Bio-Beads SM-2 Adsorbent
Chemical nature	Polystyrene-divinyl-benzene
Polarity	Nonpolar
Dipole moment	0.3 Debye
Average pore diameter (dry beads)	90 Å
Wet density (g/cc)	1.02
Capacity (Triton® X-100 detergent) g/g	0.07

Section 2
Product Description

Bio-Beads SM-2 non-polar polystyrene adsorbents are particularly useful for the adsorption of nonpolar substances or surface active agents from aqueous solutions. Holloway used Bio-Beads adsorbents to remove Triton X-100 detergent.[1] Others have since used the adsorbent for removing Triton,[2,3] as well as non-ionic detergents such as deoxycholate,[4-6] NP-40,[7] and emulgen 911.[8-10]

Bio-Beads adsorbents have been used to separate water soluble steroids,[11,12] phenols,[13] drugs,[14,15] pesticides,[16-20] trace organics,[21-23] and rhodamine.[24]

Table 2 lists some other general applications of Bio-Beads adsorbents.

Table 2. Compounds Concentrated or Separated by Bio-Beads SM Adsorbents

Trace organics	Morphine
PAH compounds	Methaqualone
Hydrocarbons and PCBs	Sulfas
Aminocarb insecticides	Free rhodamine
Carbamate insecticides	Prostaglandins
Ethyl and methyl parathion	Steroids
Metals	Bile acids
Carboxylic acids	Hormones
Phenolic acids	Purines and pyrimidines
Flavonoids	Acid dyes
Mycotoxins	Naringin and limonin
Proline and hydroxyproline	Detergents

A complete list of more than 100 applications for Bio-Beads adsorbents can be obtained by requesting technical bulletin 1461.

Section 3
Instructions for Use

3.1 Preparation of Bio-Beads SM Adsorbent (Bulk)

Due to the macroporous nature of the adsorbent, air will sometimes become trapped within the pores. As a result, it will float in solution. To correct this, slurry the adsorbent in the solution you will use later. Degas the slurry by placing it in a vacuum flask and pulling a vacuum while stirring or sonicating at the same time. Decant the excess elution solvent. The adsorbent is then ready for use, either in the batch method or column method.

3.2 Column Method

1. Slurry the Bio-Beads SM adsorbent in the solvent that will be used in the process. Usually a 66-75% slurry is necessary to fill the column. Use wet density information from Table 1 to calculate the quantity of adsorbent needed for size of the column.
2. Allow the slurry mixture to flow down the side of the column so that a minimal amount of air bubbles will be introduced into the chromatographic media bed.
3. Allow the adsorbent to settle, and elute excess solvent from the bottom of the column. Make certain that the solvent level does not go below the top of the adsorbent bed, because this will allow air bubbles to form on the top of the column.
4. Equilibrate the Bio-Beads SM adsorbent with 3 bed volumes of your desired buffer (ex. 10 mM potassium phosphate, pH 7.2).
5. Apply sample to the column.
6. Wash with 1–2 bed volumes of buffer at 0.3 ml/min.
7. The column can be regenerated by washing in bed volumes of 100% methanol, followed by rinsing in DI water.

3.3 Batch Method

The batch method is the addition of adsorbent directly into the sample followed by stirring to achieve the adsorption. This is a very simple and common method, though it requires 90–120 minutes, as opposed to 15 minutes for the column procedure.

1. Weigh 5 g of Bio-Beads SM adsorbent for every 25 ml of solution.
2. Add the adsorbent to the solution.
3. Use either a stir bar or a mechanical agitator to mix the solution at room temperature for 2 hours.
4. The sample may be recovered by decanting, or by removing it with a pipette. For smaller samples, it may be more convenient to centrifuge the sample before pipetting.

Both columns and batches can be stored. For short term storage (<1 week), use the elution solvent. For longer storage, use a 0.05% sodium azide solution or a 20% solution of an organic solvent such as methanol or isopropanol to prevent microbial growth.

Section 4
Sample Protocols

4.1 Triton X-100 Detergent Removal

Triton X-100 detergent is useful for membrane solubilization. However, the usefulness of Triton X-100 detergent has been limited by the difficulty experienced in removing the detergent from the sample. Holloway reported a simple, rapid, and mild procedure for removing Triton X-100 detergent on columns of Bio-Beads SM-2 adsorbents.[1] The following protocol is based on Holloway's published procedure. Refer to his paper for complete details.

1. Pack a 1 x 8 cm column (or 1 x 10 cm Econo-Column® chromatography column) with 5 g moist Bio-Beads SM-2 adsorbent. Equilibrate with 10 mM potassium phosphate, pH 7.2, at 4 °C. (1 g of Bio-Beads SM-2 adsorbent will adsorb 0.07 g of Triton X-100 detergent.)
2. Layer a 1 ml sample of protein solution onto the top of the bed.

3. Wash with 10 mM phosphate buffer at 0.03 ml/min. Collect fractions and assay for protein.

The column can be regenerated by washing in 4 bed volumes of methanol, followed by rinsing in DI water.

4.2 Adsorption of Unconjugated Fluorescent Dye

This procedure was used by Spack et al. to reduce high background fluorescence with rhodamine-labeled antibodies.[24] It should be useful for many fluorescent labels. The Bio-Beads SM-2 adsorbent was prewashed with 2–3 bed volumes of methanol, distilled water, and PBS, and stored in PBS until use. If trapped air within the pores of the Bio-Beads adsorbent causes the adsorbent to float, the slurried beads may be degassed to correct this.

4.3 Batch Protocol

1. Add 0.1 g of Bio-Beads adsorbent to 1–2 ml of solution containing the fluorescent-labeled antibody.

2. Incubate the beads with the solution at room temperature on a rotary platform to insure thorough mixing. Stir for 30 minutes.
3. Centrifuge the mixture, and pipette out the supernatent.
4. Free fluorescent dye will reappear upon storage, so samples should be adsorbed with the Bio-Beads SM-2 adsorbent an hour before use in immunofluorescent staining experiments.

4.4 Column Protocol

1. Slurry 2 g of Bio-Beads SM-2 adsorbent in phosphate buffer.
2. Pour a 0.8 x 4 cm Poly-Prep® column containing a 1 ml bed volume of Bio-Beads adsorbent.
3. Apply 1–2 ml solution containing the fluorescent-labeled antibody, and collect the effluent.
4. Wash the column with PBS and collect the effluent.

Section 5
Storage

All Bio-Beads SM adsorbents are stable for 5 years when stored at room temperature in the unopened container.

Section 6
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Section 7
Assistance

For help in developing your application, contact your local Bio-Rad representative, or in the U.S., call our technical service hotline at 1-800-4BIO-RAD. Request bulletin 1461 for a complete application bibliography.

Section 8
Product Information

Catalog Number	Product Description
152-3920	**Bio-Beads SM-2 Adsorbent,** 20–50 mesh, 100 g
152-8920	**Biotechnology Grade Bio-Beads SM-2 Adsorbent,** 20–50 mesh, 25 g