AG® 501-X8 and Bio-Rex® MSZ 501(D) Mixed Bed Resin

Instruction Manual

BIO-RAD

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LIT205 Rev B

Introduction

Mixed bed resins are used for deionizing water or other non-ionic substances such as urea, acrylamide, formamide, or glyoxal. Deionization is the complete removal of all ionic species from a solution.

Technical Description

AG 501-X8 mixed bed resin for deionization consists of equivalent amounts of AG 50W-X8 strong cation exchange resin H^+ form and AG 1-X8 strong anion exchange resin OH^- form. Bio-Rex MSZ 501(D) resin is similar to AG 501-X8 resin, but the uniform bead size minimizes pressure drops, increases resin capacity, and allows shorter regeneration times. Molecular Biology Grade AG 501-X8 resin is certified to be endo- and exonuclease-free and to contain no ligase inhibitors. Biotechnology Grade AG 501-X8 resin is certified to contain less than 100 micro-organisms per gram. AG 501-X8(D) resin and Bio-Rex MSZ 501(D) resin have a blue dye irreversibly bound to the anion exchange resin, which turns from blue to gold when the exchange capacity is exhausted. The resin specifications are given in Table 1.

Table 1. Mixed Bed Resin Specifications

	AG 501-X8 Resin	AG 501 -X8 (D) Resin	Bio-Rex MSZ 501(D) Resin
Chemical form	H^+ & OH^-	H^+ & OH^-	H^+ & OH^-
Resin type	Mixed bed	Mixed bed	Mixed bed
Physical form	Light and dark gold beads	Blue and gold beads	Blue and brown beads
Minimum total capacity	1.5 meq/ml	1.5 meq/ml	1.5 meq/ml
Particle diameter	300-1,180 µm	300-1,180 µm	600±100 µm
Mesh size	20-50	20-50	25-35
Matrix	Styrene divinyl-benzene	Styrene divinyl-benzene	Styrene divinyl-benzene

All of the mixed bed resins are used for deionization. Deionization can be performed by exchanging the solute cations for hydrogen on the resin and the solute anions for hydroxyl on the resin. The resulting neutralization yields water.

$$\text{Resin-H} + \text{Resin-OH} + \text{NaCl} \rightarrow \text{Resin-Na} + \text{Resin-Cl} + H_2O$$

Instructions for Use

The batch method or the column method may be used to deionize a sample with mixed bed resin.

Batch Method

The batch method is the addition of resin directly into the sample followed by stirring to achieve deionization.

1. Weigh out about 5 g of fresh resin for every 100 ml of sample.
2. Add resin to sample and stir or shake for 1 hour.
3. Filter or decant sample from resin.

Note: If samples contain sugars or polyhydric alcohols, it is important to use the minimum quantity of resin required for deionization, and to remove the resin from the sample after the deionization process to minimize adsorption of the sample. If the sample contains extremely hydrophilic proteins, the resin should be placed in dialysis tubing to avoid direct contact of the resin with the sample. If the anion resin beads change from blue to gold prior to deionization, the quantity of resin should be increased.

Column Method

Use mixed bed resin in a column to deionize larger volumes (liter volumes) of mobile phase such as water. The following procedure gives guidelines for column deionization for a range of volumes; use Table 2 to select the column size and quantity of resin.

1. Select a column with a length to diameter ratio of at least 5 to 1. Refer to Table 2.
2. Weigh out approximately 0.6 grams of resin for every 1 ml of column volume.
3. Using the resin as it comes from the bottle, pour a small portion of resin into the column. Pour the column in sections, a scoop at a time, to prevent separation of the anion and cation resins. Add water, keeping about 1/4 inch of water above the resin.
4. Repeat step 3, alternating resin and water, until the total amount of resin is added. The resin will have a tiger's tail appearance; more bands will mean a higher efficiency. Remove any trapped air bubbles by tapping the column during packing.
5. When the column is packed, wash the resin with 3 bed volumes of deionized water. Discard wash.

6. Slowly pour the solution to be deionized into the reservoir above the column, then elute slowly through the column taking care not to disturb the resin bed. Discard the first 1-2 bed volumes of the solution.
7. The quality of the deionized solution may be verified by measuring its conductivity against the conductivity of the starting solution.

Table 2. Guidelines for Column Deionization

Column Size (diameter x length)	Weight of Resin (g)	Approximate Volume of Deionized Water* (liters)
0.5 x 20	2.6	1.5
1.0 x 50	27.0	6.7
1.5 x 100	118.0	29.0
2.5 x 100	325.0	80.0
5.0 x 100	1,300.0	325.0
15.0 x 120	13.6	3,500.0

* Based on using 100 ppm NaCl. This volume will vary depending on initial water quality.

The dye (D) form of resin may be used to deionize solutions until the dye begins to fade from blue to gold, indicating capacity is exhausted. In a large column, the dye will change color starting at the top of the column, and deionization will continue until the color change

occurs at the bottom of the column. If the column is not used for more than 1 week, it should be washed with 3 bed volumes of water prior to use.

Regeneration

Mixed bed resin used in laboratory-scale applications is not normally regenerated because the of the difficulty in separating the mixed anion and cation resins, the large volumes of regenerants required for the anion resin, and difficulty in accurately remixing chemically equivalent resins. The following procedure may be used and is cost-effective for large-scale applications.

To regenerate a mixed bed resin, separate the anion exchange resin from the cation exchange resin by back-washing the resin in a column. First use a low flow rate to expand the bed. This separates the bed into two regions: the anion exchanger on top and the denser cation exchanger on the bottom. Then slowly increase the flow rate to carry the anion exchanger out the top of the column and into a separate container. An alternative separation method is to shake the resin in twice its volume of water, let it settle, and decant the top layer containing the anion exchanger. This procedure is repeated until separation is complete.

Regenerate the cation exchanger using 3 bed volumes of 3 N HCl and rinse with 4 bed volumes of deionized water or until the effluent is >pH 5. Regenerate the anion exchanger with at least 10 bed volumes of 3 N NaOH and rinse to <pH 9 with deionized water. Regeneration flow rates should be about 2 ml/min/cm^2.

Mix the resins thoroughly in the original column by gently backwashing with deionized water while agitating with a stirring rod or air, then stopping the upflow and continuing to stir as the resin settles.

Shelf Life

Mixed bed ion exchange resins are stable for 2 years when stored at 21 °C and protected from exposure to ultraviolet light. The shelf life may be extended by storing the resin at 4 °C.

Applications

AG 501-X8 and Bio-Rex MSZ 501(D) mixed bed resins may be used to prepare non-ionic reagents for critical analytical applications. Either the batch or column method may be used to obtain purified urea, acrylamide,

formamide, glyoxal, or PEG, although the batch technique is much more common.

Batch Deionization of Formamide, Acrylamide, and Glyoxal

This procedure was originally described by Maniatis, et al. [Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Lab (1982)] for deionization of formamide, and can be used for any nonionic reagent such as acrylamide, glyoxal, urea, or water.

1. Weigh 5 grams of resin for every 100 ml of formamide or acrylamide solution to be deionized. For glyoxal, use 1 gram of resin per ml of glyoxal. This quantity of resin will be sufficient for any concentration of these solutions.
2. Wash resin briefly with the solution to be deionized, using about 1 ml solution per ml of resin. Discard the solution.
3. Add resin to sample and stir for 1 hour. Check pH with pH paper to insure deionization is complete. Repeat Step 3 using new resin if necessary.
4. Filter or decant sample from resin.

Note: Formamide interferes with the color change of the dye, but will not affect the deionization capacity of the resin.

Batch vs. Column Deionization of Urea

Table 3 compares deionizing urea both by the batch and the column technique. The decrease in conductivity using the batch method is plotted in Figure 1.

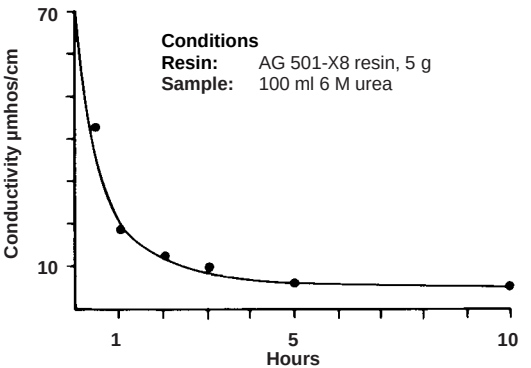

Fig. 1. Decrease in conductivity using batch method.

Table 3. Deionization of Urea by Batch and Column Methods

	Batch	Column
Sample	100 ml 6 M urea	100 ml 6 M urea
Starting conductivity	70 µmho/cm	70 µmho/cm
Amount of mixed bed resin	5 grams	5 grams (~8 ml)
Final conductivity	5.0 µmho/cm	0.2 µmho/cm
Time	~5 hours	~10 minutes

Ampholyte Removal

Carrier ampholytes may be quantitatively removed from protein fractions derived from isoelectric focusing using mixed bed resin. Mixed bed ion exchange chromatography represents a method for the quantitative removal of carrier ampholytes. Figure 2 illustrates the separation of proteins from ampholytes and sucrose. A 3 ml sample was applied to a 0.9 x 25 cm column of AG 501-X8 resin. AG 501-X8 resin has also been shown to be useful for separating peptides of greater than 4,000 daltons from ampholytes.

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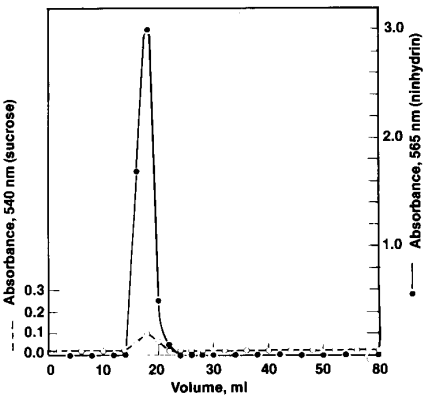

Fig. 2. Separation of hemoglobin from ampholytes and sucrose on a mixed bed ion exchange column (AG 501-X8 resin). A hemoglobin blank in the sucrose determination gives an absorbance of about 0.1. Neither sucrose nor ampholyte emerged from the column in the total volume tested.[3]

References

1. Kapp, O. H. and Vinogradov, S. N., *Anal. Biochem.*, **91**, 230 (1978).
2. Bakker, J. A., Vanden Brande, J. L. and Haggerbrugge, C. M., *J. Chromatog.*, **209**, 273 (1971).
3. Brown, W. D. and Green, S., *Anal. Biochem.*, **34**, 593 (1970).

Product Information

Catalog Number	Description	Mesh Size	Diameter (µm)	Nominal Density (g/ml)	Pkg. Size
142-6424	**AG 501-X8 Resin**	20-50	300-1,180	0.75	500 g
142-6425	**AG 501-X8 (D) Resin**	20-50	300-1,180	0.75	500 g
142-7425	**Bio-Rex MSZ 501 (D) Resin**	25-35	500-700	0.75	500 g
143-7424	**Biotechnology Grade AG 501-X8 Resin**	20-50	300-1,180	0.75	100 g
143-7425	**Biotechnology Grade AG 501-X8 (D) Resin**	20-50	300-1,180	0.75	100 g
143-6424	**Molecular Biology Grade AG 501-X8 Resin**	20-50	300-1,180	0.75	100 g
143-6425	**Molecular Biology Grade AG 501-X8 (D) Resin**	20-50	300-1,180	0.75	100 g