Bio-Rex® Weakly Acidic Cation Exchange Resin

Instruction Manual

Catalog Numbers
142-5822, 142-5832,
142-5842, 142-5852,
143-5832, 143-5852

BIO-RAD

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Section 1
Introduction

Bio-Rex 70 resin is a weakly acidic cation exchanger. This resin is used for the purification and fractionation of peptides, proteins, antibiotics, and other cationic molecules.

Section 2
Technical Description

Bio-Rex 70 resin is available as both Analytical Grade and Biotechnology Grade resin. The Analytical Grade Bio-Rex 70 resin has been exhaustively sized, purified, and converted to make it suitable for accurate, reproducible analytical techniques. Biotechnology Grade Bio-Rex 70 resin is an Analytical Grade resin which is certified to contain less than 100 microorganisms per gram of resin.

The resin contains carboxylic acid exchange groups on a macroreticular acrylic polymer lattice. The high porosity of the resin allows large protein molecules to penetrate the pores and have access to the exchange sites located throughout the matrix. In an ion exchange procedure the counterions on the resin are replaced by

sample ions that have the same charge. On a cation exchange resin such as Bio-Rex 70 resin, neutral molecules and anions do not interact with the resin.

Normally Bio-Rex 70 resin is supplied in the sodium form, but the resin can be converted from one ionic form to another. Usually the resin is used in an ionic form with a lower selectivity for the functional group than the sample ions to be exchanged. The sample ions are then exchanged when introduced, and can be eluted by introducing an ion with higher affinity for the resin or a high concentration of an ion with equivalent or lower affinity. Table 1 shows the relative selectivity of various monovalent and divalent counterions, as well as some nominal properties of the Bio-Rex 70 resin.

Table 1. Properties of Bio-Rex 70 Resin

Wet density	0.6 ml/g (Na^+ and H^+ forms)
% moisture	65-74% (Na^+ form); 55-65% (H+ form)
Swelling	1 ml (H^+ form); 1.7 ml (Na+ form)
Capacity	10 meq/dry g
Na+ form	0.5 meq/ml
H+ form	2.4 meq/ml
Pore volume	0.10 cc/cc
Pore size range	700-4,000 Å
Thermal stability	Up to 100 °C
Normal operating pH range	5 to 14
Order of selectivity for monovalent ions	H>>Ag>K>Na>Li
Order of selectivity for divalent ions	H>>Fe>Ba>Sr>Ca>Mg

Bio-Rex 70 resin has a high capacity for macromolecules, yet does not bind proteins so tightly that they are difficult to elute. The acrylic polymer matrix is hydrophilic, and generally free of nonspecific binding or denaturing effects on proteins. Figure 1 shows the titration curve. Note that the optimal operating pH range is above pH 5.

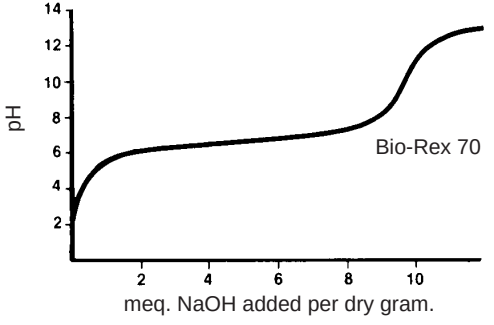

Fig. 1. Bio-Rex 70 titration curve.

Bio-Rex 70 resin is available in several particle size ranges. The flow rate in a chromatographic column increases with increasing particle size. However, the attainable resolution increases with decreasing particle size and narrower size distribution ranges. Particle size is given either in mesh size or micron size. Mesh refers to the number of openings per inch on the screens used to size ion exchange resins. Therefore, the larger the mesh size, the smaller the particle size. Table 2 shows wet mesh and equivalent micron diameters.

Table 2. Wet Mesh and Equivalent Micron Diameters

Wet Mesh											
(U.S. Standard)	16	20	40	50	80	100	140	200	270	325	400
Micron Diameter											
(1 µ=0.001 mm)	1190	840	420	297	177	149	105	74	53	44	37

Large mesh material (20-50 mesh and 50-100 mesh) is used primarily for large preparative applications, and batch operations where the resin and sample are slurried together. Medium mesh resin (100-200 mesh) is used primarily in column chromatography, for analytical and laboratory scale preparative applications. Fine mesh material (200-400 mesh and minus 400 mesh) is used for high resolution analytical separations.

Section 3
Resin Equilibration

Especially when Bio-Rex 70 resin is used to separate multi-component samples, it is very important to equilibrate the resin to the initial buffer conditions before the sample is applied. In general, unequilibrated resin will not give reproducible results. Due to its weakly acidic functional groups. Bio-Rex 70 resin is slow to equilibrate. The following procedure is recommended:

1. Place the Bio-Rex 70 resin in a beaker with the buffer in which it is to be equilibrated. The volume of buffer should be four to five times the volume of the resin.
2. Allow the resin to equilibrate for at least 30 minutes. Adjust the pH with acid or base. Re-equilibrate. Repeat until pH is stable.
3. When pH is stable, decant the buffer off and repeat steps 1 and 2 using fresh buffer. Repeat until no pH change is noted when fresh buffer is added. This may take several buffer changes.
4. Decant excess buffer off and pour the resin into the column. Pass 2-3 bed volumes of buffer through the column, monitoring both pH and conductivity. When the pH and conductivity of the effluent are the same as that of the influent, the resin is fully equilibrated and ready for sample application.

Section 4
Resin Conversion

Bio-Rex 70 resin can be converted to other ionic forms by washing with a 0.5-1.0 M solution of the desired counterion. Conversion to a counterion of relatively high selectivity (see Table 1) will be rapid (2-3 bed volumes), while conversion to a counterion of relatively low selectivity will be slower (3-5 bed volumes). Conversion is complete when the starting counterion is no longer detected in the effluent. In most cases, this can be monitored by pH or simple qualitative tests.

Section 5
Instructions for Use

Bio-Rex 70 resin may be used with either the batch method or the column method. The batch method consists of adding the resin directly to the sample and stirring. The column method requires preparing a column filled with resin, and passing the sample through.

5.1 Batch Method

The batch method is performed by adding the resin directly to the sample and stirring. The resin should be in correct ionic form and equilibrated prior to beginning.

1. Weigh out about 5 grams of resin for every 100 ml of sample. For larger scale applications or when an exact amount of resin is needed, calculate the resin volume based on the resin capacity.
2. Add resin to the sample and stir or shake (gently) for 1 hour.
3. Filter or decant the sample from the resin.

5.2 Column Method

The column method involves pouring a column of resin and passing the sample through to achieve the separation. Particle size will determine the flow rate, which will affect the separation. The resin should be in the correct ionic form and equilibrated prior to adding the sample.

1. Calculate the amount of resin required based on the expected resin capacity and sample concentration. If the sample ionic concentration is unknown, begin with 5 grams of resin for 100 ml of sample, and then optimize the volumes after obtaining the results.
2. Insure that the resin is in the proper ionic form, which will allow the sample ions to be exchanged onto the resin. If conversion of the resin to another ionic form is necessary, use the guidelines described in Section 4 for resin conversion.
3. Prepare the initial buffer, so that the pH and ionic concentration will allow the sample ions to be exchanged on to the column. For unknown solutions, use deionized water.
4. Slurry and pour the resin into the column. Resin should be equilibrated according to the instructions outlined above. Poorly equilibrated resin will not give reproducible results.
5. Allow excess buffer to pass through the column, leaving enough buffer to just cover the top of the resin bed.
6. Apply the sample dropwise to the top of the column without disturbing the resin bed. Drain the sample into the top of the bed and apply several small portions of starting eluant, being very careful to rinse down the sides of the column and to avoid stirring up the bed. Drain each portion to the level of the resin bed before

the next portion is added. Never allow the liquid level to drain below the top of the resin bed sample.

7. The actual flow rate that is used will depend upon the application, the resin, and the column cross section. To obtain flow rates for any given size column, multiply the suggested flow rates in Table 3 by the column cross-sectional area. Table 3 gives typical flow rates of analytical grade resins.
8. If a cation-free solution is the goal, collect the effluent. If the concentrated cations are of interest, allow all of the sample to pass through the column, then elute the sample with a solution containing a counterion of higher selectivity than the bound cation.

Table 3. Suggested Flow Rates for Ion Exchange Resin Columns

Application	Flow Rates ml/min/cm^2
Removing trace ions	5-10
Separations with very few components	1-3
Separations of multi-component samples	0.3-1.0
Using high resolution resins with small particle size	0.1-0.2

Section 6
Resin Regeneration

Bio-Rex 70 resin is most efficiently regenerated in a column. Regenerate to the sodium form by washing with 3 bed volumes of 0.5 N NaOH. The flow rate should not exceed 1 ml/min per cm2 cross-sectional area of the column. Conversion is complete when the pH becomes greater than 9. Rinse with 4 bed volumes of deionized water and equilibrate according to the procedure above. Remember that the resin volume will approximately double when converting from the hydrogen form to the sodium form.

Section 7
Applications

Bio-Rex 70 resin has been used for the purification and fractionation of enzymes, histones, endonucleases, and toxins. Table 4 lists a variety of applications using Bio-Rex 70 resin.

7.1 Protein, Peptide, and Amino Acid Separations

Proteins may be separated from one another using the macroporous Bio-Rex 70 cation exchange resin, because this resin has pores of sufficient size to admit the proteins. The effective exclusion limit of this resin has not been established, however, from the many separations reported, the limit exceeds 75,000 daltons.

Table 4. Applications of Bio-Rex 70 Resin

Purification of the rep protein of *E. coli*	Scott, J. F. and Kornberg, A., *J. Biol. Chem.*, **253,** 3292 (1978).
Purification of human saliva lysozyme	Vasstrand, E. N. and Jensen, H. B., *Scand. J. Dent. Res.*, **88,** 219 (1980).
Purification of neurotoxins from sea snake venom	Karlsson, E., Eaker, D., Fryklund, L. and Kadin, S., *Biochemistry*, **11,** 4628 (1972).
Purification of initiation factor 2 from calf liver	Stringer, E. A., Chaudhuri, A. and Maitra, U., *J. Biol. Chem.*, **254,** 6845 (1979).
Purification of histones	D'Anna, J. A., Strniste, G. F. and Gurley, L. R., *Biochemistry*, **18,** 943 (1979); Thompson, J. A., Stein, J. L., Kleinsmith, L. J. and Stein, G. S., *Science*, **194,** 428 (1976).
Purification of restriction endonucleases	Green, P. J., et al., *Nucleic Acids Research*, **5,** 2373 (1978).
Purification of human epidermal growth factor	Savage, C. R. Jr. and Harper, R., *Anal. Biochem.*, **111,** 195 (1981).
Purification of human C1q	Tenner, A. J., Lasavre, P H. and Cooper, N. R., *J. Immunol.*, **127,** 648 (1981).

Table 4. (Continued)

Purification of inhibitory protein of human erythrocytes	Fearon, D. T., *Proc. Nat. Acad. Sci. USA*, **76,** 5867 (1979).
Measurement of glycosylated hemoglobin	Trivelli, L. A., Ranney, H. M. and Lai, H. T., *N. E. J. Med.*, **284,** 353 (1971); Schifreen, R. S., Hickingbotham, J. M. and Bowers, G. N. Jr., *Clin. Chem.*, **26,** 466 (1980).
Purification of protein allergens of white-faced yellow hornet, and yellow jacket venoms	King, T. P., Sobotka, A. K., Alagon, A., Kochoumiam, L. and Lichtenstein, L. M., *Biochemistry*, **17,** 5165 (1978).
Purification of oximdolealanine -62 lysozyme	Shrake, A. and Rupley, J. A., *Biochemistry*, **19,** 4044 (1980).
Isolation of gentamicin from serum	Habbal, Z. M., *Clin. Chim. Acta*, **95,** 301 (1979).
Isolation of brain histamine	Lewis, S. J., Fennessy, M. R., Laska, F. J. and Taylor, D. A., *Agents and Actions*, **10,** 197 (1980).
Determination of thiamine in food	Ellefson, W. C., Richter, E., Adams, M. and Baillies, N. T., *J.A.O.A.C.*, **64,** 1336 (1981).
Purification of toxins	Levinson, S. R., Curatolo, C. J., Reed, J. and Raftery, M. A., *Anal. Biochem.*, **99,** 72 (1979).
Isolation of siderophores from fungus	Frederick, C. B., Szaniszlo, P. J., Vickrey, P. E., Bentley, M. D. and Shive, W., *Biochemistry*, **20,** 2432 (1981).
Purification of *E. coli* photoreactivity enzyme	Koka, P., *Biochemistry*, **20,** 2914 (1984).
Purification of polypeptides from croatalus atrox venom	Hamilton, S. L., Yatani, A. and Hawkes, M. J., et al., *Science*, **229,** 182 (1985).
Purification of a yeast DNA repair enzyme	Johnson, A. W. and Demple, B., J. *Biol. Chem.*, **263,** 18009 (1988).

Table 4. (Continued)

DNA A protein of *E. coli*	Sedimizu, K., *et al.,* J. *Biol. Chem.,* **263,** 7136 (1988)
Hemoglobin from blood	Ersser, R. S., *et al., Biomedical Chrom.,* **1,** 183 (1986).
Peptides derived from proenkephalin A	Wilson, S. P., *J. neurosci. Methods,* **15,** 155 (1985).
Hemocyanin	Moore, M. D., *et al., J. Biol. Chem.,* **261,** 10511 (1986).
Urogastrone	Savage, C. R. and Harper, R., *Anal. Biochem.,* **111,** 195 (1981).
Lysozyme	Matei, L., *Rev. Roium. Biochem.,* **23,** 45 (1986).
Nonhistone chromosomal proteins	Wen, L. and Reeck, G. R., *J. Chromatog.,* **314,** 436 (1984).
Catabolite gene activator proteins of *E. coli*	Blazy, B. and Ullmann, A., *J. Biol. Chem.,* **261,** 11645 (1986).
FLP recombinase protein from *S. cerevisiae*	Bruckner, R. C. and Cox, M. M., *J. Biol. Chem.,* **261,** 11798 (1986).
Amine and amino acid determination	Carlucci, F. V. and Karmas, E., *J. A. O. A. C.,* **71,** 564 (1988).
Alpha and beta subunits of R-phycocyanin II	Ong, L. J. and Glazer, A. N., *J Biol. Chem.,* **262,** 6323 (1987).
Extraction of GIIIA	Cruz, L. J., et al., *Biochem.,* **28,** 3437 (1989).

Section 8
Product Information

Catolog Number	Dry Mesh Designation	Ionic Form	Pkg. Size	Minimum Wet Capacity (meq/ml)	Diameter (microns)	Density (Nominal) gm/ml
Analytical Grade Bio-Rex 70 Resin						
142-5822	20-50	Sodium	500 g	2.4	300-1,180	0.70
142-5832	50-100	Sodium	500 g	2.4	150-300	0.70
142-5842	100-200	Sodium	500 g	2.4	75-150	0.70
142-5852	200-400	Sodium	500 g	2.4	45-75	0.70
Biotechnology Grade Bio-Rex 70 Resin						
142-5832	50-100	Sodium	500 g	2.4	150-300	0.70
142-5852	200-400	Sodium	500 g	2.4	45-75	0.70

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547
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