AG® 3, AG 4, and Bio-Rex® 5 Anion Exchange Resin

Instruction Manual

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LIT207 Rev B

AG 3, AG 4, and Bio-Rex 5 Resin Weakly Basic and Intermediate Basic Anion Exchange Resin Instructions

Introduction

AG 3-X4 and AG 4-X4 resins are weakly basic anion exchangers. These resins can exchange anions, such as organic acids, with weak interactions, allowing easy elution and regeneration. They will also adsorb mineral acids, such as hydrochloric acid and perchloric acid, to yield a neutralized solution. Bio-Rex 5 resin is an intermediate basic anion exchanger, which also exchanges anions, such as iodide, bromide, and chloride, with weak interactions, allowing easy elution and regeneration.

Technical Description

AG 3-X4, AG 4-X4, and Bio-Rex 5 resins are all analytical grade purity. They have been exhaustively sized, purified, and converted to make them suitable for accurate, reproducible analytical techniques. AG 4-X4 resin is also available in Biotechnology Grade, Analytical Garde resin which has been further processed

and is certified to contain less than 100 microorganisms per gram of resin.

AG 3-X4 resin and AG 4-X4 resin are both 4% crosslinked and have a tertiary amino functional group. The resins differ in their backbone resin matrix material. AG 3-X4 resin has a styrene-divinylbenzene copolymer lattice matrix, and AG 4-X4 resin has an acrylic matrix. These two matrices differ in their physical and mechanical strength, and in their inherent hydrophilic nature. The styrene-divinylbenzene matrix (AG 3-X4 resin) is very strong mechanically, but is hydrophobic, which can cause adsorption of large organic molecules or proteins. The acrylic matrix (AG 4-X4 resin) is softer, and therefore sensitive to crushing from excess flow rates or very large column sizes. This material is hydrophilic and is suitable for use with high molecular weight organic compounds, proteins, and carbohydrates.

Bio-Rex 5 intermediate base resin contains primarily tertiary, but also some quaternary, amines on a styrene-divinylbenzene lattice. Bio-Rex 5 resin can be used to separate organic acids from neutral sugars. When the resin is regenerated with base, the quaternary ammonium

groups will be in the hydroxide form, while the tertiary amine groups will be in the free base form.

The physical properties of the resins are listed in Table 1. These resins are thermally stable and resistant to solvents (alcohols, hydrocarbons, etc).

Table 1. Summary of Properties

Resin Type	Active Group	Order of Selectivity	Thermal Stability	Solvent Stability
Bio-Rex 5 intermediate base anion exchanger	$R\text{-}N(CH_3)_2$	$I>phenolate>HSO_4>ClO_3>NO_3>Br>CN>HSO_3>NO_2>Cl>HCO_3>IO_3>H_2COO>HC>OH>F$	Good to 60 °C	Good
AG 4-X4 AG 3-X4 weakly basic anion exchangers	$R\text{-}CH_2N+(CH_3)_2$	$HSO_3>HCit>CrO_3>H_2SO_4>$ tartaric>oxalic $>H_3PO_4>H_4AsO_4>HNO_3>HI>HBr>HCl>HF>HCOO>HAc>H_2CO_3$	Good to 60 °C	Good

Mechanism

Both AG 3-X4 and AG 4-X4 resin are available in the free base form. Here the functional group is neutral, and is not charged. When a mineral acid is passed over

the resin, the nitrogen in the functional group becomes protonated, allowing the adsorption of the mineral acid. When either of these two resins is washed with base, the resin goes back into the free base form. Figure 1 shows the adsorption of hydrochloric acid onto the resin.

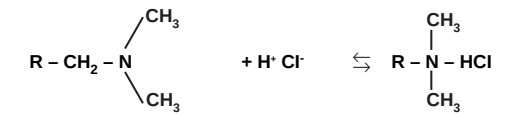

Fig. 1. Adsorption of hydrochloric acid.

Weakly basic anion exchangers, such as AG 3-X4 and AG 4-X4 resins, will act as anion exchangers when equilibrated and used at or below pH 7. In an ion exchange procedure, the counterions on the resin are replaced by sample ions that have the same charge. The functional group on these resins is a tertiary amine, which will be positively charged at or below pH 7. The corresponding anion is the counterion which will be

exchanged with the anion in the sample. Neutral compounds and cations will not interact with the resin, and should pass through in the void volume. A 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 onto the resin 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 counterions. In general, the lower the selectivity of the counterion, the more readily it exchanges for another ion of like charge. The order of selectivity can also be used to estimate the effectiveness for different ions as eluants, with the most highly selective being the most efficient. To convert from a highly selected to a less highly selected form requires an excess of the new ion. Table 2 outlines common techniques for converting ion exchange resins from one ionic form to another. Resin conversion is most efficiently carried out in the column mode.

Table 2. Techniques for Resin Conversion

Resin:	AG 4-X4 resin AG 3-X4 resin Bio-Rex 5 resin
Conversion from(1): Cl^-	→ free base+OH^-
Reagent used:	0.5 NaOH(2)
Volumes of solution per volume of resin:	2
Flow rate(3) **ml/min/cm² of bed**:	1
Test for completeness of conversion:	Cl^-(4)
Rinse: volume DI water per volume resin:	4
Test for completion of rinsing:	pH <9

(1) Typical conversions are listed. The same reagents can be used to convert from other ionic forms. Two step regeneration, ion exchange followed by neutralization, is included because of ease of conversion and savings on expensive reagents.

(2) Use U.S.P. or C.P. grade (low chloride).

(3) For 50-100 or finer mesh resin. For 20-50 mesh, about 1/5 the flow rate is recommended.

(4) Test for Cl^- in effluent: Acidify sample with a few drops of conc. HNO_3. Add 1% $AgNO_3$ solution. White ppt indicates Cl^-, yellow Br^- or too basic.

All three resins are 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 number, the smaller the particle size. Table 3 shows wet mesh and equivalent micron diameters.

Table 3. Wet Mesh and Equivalent Micron Diameters

Wet mesh (U.S. Standard)	16	20	40	50	80	100	140	200	270	325	400
Micron diameter	1,190	840	420	297	177	149	106	74	53	44	37

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

Instructions for Use

AG 3-X4, AG 4-X4, and Bio-Rex 5 resin may be used in 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.

Batch Method Instructions

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

Column Method Instructions

The column method involves pouring a column with the 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 ionic concentration of the sample is unknown, begin with 5 grams of resin for 100 ml of sample, and 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 in Table 2 for resin conversion.
3. Prepare the initial buffer, so that the pH and ionic concentration will allow the sample ions to be exchanged onto the column. For unknown solutions, use deionized water.
4. Slurry and pour the resin into the column. Equilibrate the resin in the initial buffer using 3 bed volumes of buffer. Poorly equilibrated resin will not give reproducible results.

Alternatively, equilibration can be done by the batch technique, prior to pouring the column. First, convert the resin to the appropriate form, then suspend it in the starting buffer. Check the pH with a pH meter while stirring continuously. Adjust the pH by adding acid or base dropwise to the buffer until the desired pH is obtained. Then transfer the resin to the column and pass 1 bed volume of starting buffer through the column.

5. Slurry the resin in the initial buffer and pour the column. Allow excess buffer to pass through the column, leaving just enough buffer to 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.
7. The actual flow rate that is used will depend upon the application, the resin, and the column cross section. Table 4 gives typical flow rates of analytical grade resins.
8. If an anion free solution is the goal, collect the effluent. If the concentrated anions are of interest, allow all of the sample to pass through the column. Then, elute the anions from the resin with a solution containing a counterion of higher selectivity than the bound anion.

Table 4. Suggested Flow Rates for Ion Exchange Resin Columns

Application	Flow Rate 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

Sample Protocol

Separating Neutral Sugars and Acids from Wine and Grape Must Samples

This procedure describes a method for separating organic acids and neutral sugars using Bio-Rex 5 resin, according to the protocol described by McCord, *et al.*[1]

Since some of these compounds co-elute during HPLC, this simple fractionation technique was developed.

Protocol

1. In a test tube, carefully pipet:

   1.00 ml wine or grape must

   0.100 ml 5% formic acid

   0.100 ml 5% propylene glycol

   0.2 ml 5 N ammonium hydroxide

   Mix well. Insure that the pH is between 8 and 9; adjust if necessary. Keep closed.
2. Prepare the column as follows (do not let the column dry out):
   a. Slurry 1 g Bio-Rex 5 100-200 mesh resin (Cl^- form) in 3 ml water.
   b. Pour slurry into a polypropylene column.
   c. Using a syringe, expel excess liquid, then wash bed with 5 ml water.

3. Neutral fraction.
   a. Collect in a clean vial (scintillation vials work well) and use a syringe to hasten flow through the column.
   b. Add the sample to column, drain through.
   c. Wash with water (1 ml, 4 ml, 4 ml).
   d. Cap and mix well. Store at 4 °C.
4. Acid fraction.
   a. Change to a fresh vial.
   b. Carefully add 1 ml of 10% sulfuric acid.
   c. Wash with water (1 ml, 4 ml, 4 ml).
   d. Cap and mix well.

Technical Information

If you have any questions or need additional information, contact your local Bio-Rad representative.

Reference

1. McCord, J. D., Trousdale, E. and Ryu, D. D. Y., *Am. J. Enol. Vitic.*, **35**, 28 (1984).

Product Information

Catalog Number	Description	Mesh Size	Ionic Form	Pkg Size	Minimum Wet Capacity meq/ml	Nominal Diameter (microns)	Density g/ml
140-4341	AG 4-X4 Resin, Analytical Grade	100-200	Free Base	500 g	0.8	75-150	0.70
140-5341	AG 3-X4 Resin, Analytical Grade	100-200	Free Base	500 g	1.0	106-250	0.70
140-7841	Bio-Rex 5 Resin, Analytical Grade	100-200	Chloride	500 g	1.1	75-150	0.70
140-7851	Bio-Rex 5 Resin, Analytical Grade	200-400	Chloride	500 g	1.1	45-75	0.70
143-3341	AG 4-X4 Resin, Biotechnology Grade	100-200	Free Base	100 g	0.8	75-150	0.70