Analytical Grade AG 11® A8 Ion Retardation Resin

Instruction Manual

Catalog Number 732-2032

BIO-RAD

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Section 1
Introduction

AG 11 A8 ion retardation resin is extremely useful for chromatographic desalting, or removing acid from non-ionic molecules. It separates salts from organic materials by absorbing both anions and cations in equivalent amounts while allowing the organic compounds to pass through. Both Analytical Grade and Biotechnology Grade AG 11 A8 resin is available. Biotechnology Grade 11 A8 resin is certified to contain less than 100 microorganisms per gram of resin. The AG 11 A8 resin selectively retards ionic substances, so it can be used not only for desalting non-ionic solutions, but also for separating ions from ionic substances. Organic molecules, even ionic species such as acidic and basic amino acids, are usually not absorbed by AG 11 A8 resin, whereas inorganic ions are absorbed, thus achieving the separation.

Section 2
Technical Description

AG 11 A8 resin is made by polymerizing acrylic acid inside AG 1-X8 resin to produce a spherical resin bead containing paired anion and cation exchange sites. The result is a styrene divinylbenzene crosslinked, rigid polymer lattice with attached quaternary ammonium groups (strongly basic anion exchange groups) within which weaves a trapped, linear, relatively flexible acrylic polymer having carboxyl groups (weakly acidic cation exchange groups). Each resin bead is a molecular mixture of a cation and an ion exchanger. When the resin is free of absorbed salts, the self-absorbed form, each group is the counterion of the other form. Figure 1 shows the self-absorbed form of the resin.

Ion retardation occurs due to the unique structure of the resin with its adjacent anion and cation exchange sites. These sites attract mobile anions and cations via weak attractions. The mechanism is absorption rather than the conventional ion exchange. The figure below shows the absorption of NaCl:

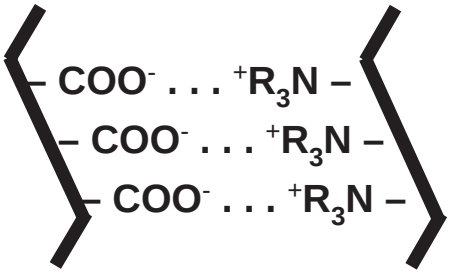

Fig. 1. Structure of AG 11 A8 resin in the self absorbed form. The solid curved lines represent the organic polymer lattice. The dotted lines represent ionic bonds.

ø – COO^- . . . . ^+R_3N – ø = NaCl " ø – $COONaClR_3N$ – ø

Absorbed salts move down the column during water elution because the fixed exchange groups compete with the mobile salt ions to become self-absorbed.

ø – $COONaClR_3N$ – ø ” ø – COO^- . . . . ^+R_3N – ø = NaCl

A flow of water washes away the mobile ions, driving the reaction to the right. As the salts move down the column, they are repeatedly absorbed and desorbed, and thus retarded.

The strength with which AG 11 A8 resin absorbs an ion varies with the type of ion. Table 1 shows the selectivity of some monovalent and divalent ions on

AG 11 A8 resin. The strength with which a salt is absorbed, and therefore the degree to which it is retarded and separated from organic substances, is proportional to the strength with which one or both of its constituent ions is absorbed.

Table 1. Order of Selectivity for Monovalent and Divalent Ions

Anions	I > phenolate > HSO_4 > ClO_3 > NO_3 > Br > CN > HSO_3 > NO_2 > Cl > HCO_3 > IO_3 > H_2COO > Ac > OH > F
Cations	H> Ag > Rb > Cs > K > NH_4 > Na > Li > Zn > Cu > Ni > Co

The hydrogen ion is held so strongly that it is not eluted with water. This makes AG 11 A8 resin excellent for rapid quantitative removal of acids.

Section 3
Instructions for Use

3.1 General Protocol

A typical procedure with AG 11 A8 resin consists of four steps.

1. Slurry about 30 grams of AG 11 A8 resin and pour into a column to give a bed of 1 x 40 cm.
2. Add 4 ml of sample onto the bed and continue at a linear flow rate of 0.3 cm/min.
3. Elute with deionized water at the same flow rate, collecting 4 ml fractions.
4. Continue elution until the effluent is salt free.

Note: Occasionally, the AG 11A8 resin will clump together in deionized water due to the paired anion and cation exchange sites of the resin. In order to eliminate clumping, the column should be packed in a salt solution (refer to Alternate Protocol).

3.2 Alternate Protocol

1. Slurry about 30 grams of AG 11 A8 resin in twice the volume of 0.5M NaCl. Gently pour the resin into a 1 x 40 cm column.
2. Wash the column with 3 bed volumes of 0.5 M NaCl.
3. Wash the column with at least 10 bed volumes of deionized water at 0.3 cm/min.
4. Add 4 ml of sample onto the bed and continue at a linear flow rate of 0.3 cm/min.
5. Elute with deionized water at the same flow rate, collecting 4 ml fractions.
4. Continue elution until the effluent is salt free

Section 4
Optimizing Results

Resolution, recovery, and speed cannot all be at a maximum in any one operation. Each of these factors is affected by column length, loading, flow rate, temperature, and feed-solution composition. Any procedure using AG 11 A8 resin should be designed with

these effects in mind. Table 2 gives the normal range of operating conditions on AG 11 A8 resin.

Table 2. Operating Conditions

	Normal Range	Suggested*
Column length (cm)	10–80	40
Loading (percent)**	5–90	10
Flow rate (cm/min)	0.1–4	0.3
Temperature (°C)	5–80	room temp
Composition of Feed		
Protein (mg/ml)	10^{-6}–100	any value
Amino acid (molarity)	10^{-4}–1	any value
Salt (molarity)	10^{-3}–3	less than 1
Acid (molarity)	10^{-4}–6	any value

* These values are recommended for initial tests of the desalting method. They are not necessarily optimum.

** Loading is the volume of sample expressed as a percentage of the resin bed volume: 4 ml of sample on a 40 ml bed is a 10% loading.

4.1 Resolution

Resolution of the salts and organic components is increased with increased column length, temperature, and affinity of the salt ions for the absorption sites, and with

decreased loading and flow rate. Retardation, and therefore resolution, requires that the ions diffuse into the resin bead (flow rate, temperature), find an unloaded site (loading, column length), and associate with that site (affinity).

4.2 Recovery

Recovery after an ion retardation procedure is often 100%. However, some losses may be encountered due to adsorption or deactivation on the resin's exchange sites. Increased loading and flow rate, or decreased column length, may, in such cases, improve recovery. Recovery may also be improved by preconditioning the column with alternate cycles of sample solution and water elution until the resin has reached equilibrium.

4.3 Speed

Speed is increased with increased flow rate and temperature, and with decreased column length and sample load.

Section 5
Resin Regeneration

AG 11 A8 resin may usually be used repeatedly without special regeneration, water elution simply being continued until the effluent is salt-free. In some cases, the resin may retain an organic or salt component. Regeneration may be accomplished using a concentrated salt solution followed by a water wash (see Table 3). Due to the high affinity of the carboxyl group for the hydrogen ion, a regeneration procedure using acid is even more effective than one using salt. However, the acid must be followed by a neutralization step to remove absorbed hydrogen ions. Likewise, if large quantities of an acidic solution (pH<4) have been desalted, a neutralization step may eventually be necessary to restore the original high desalting capacity (start with step A2, Table 3). Any salt or acid may be used, but the procedure given in Table 3 will return the resin to the form in which it was shipped.

Table 3. Regeneration Procedure

Type of Regeneration	Step	Reagent	Quantity (bed volumes)	Linear Flow rate (cm/min)
Salt	S1	1 M NH_4Cl	5 *	2
	S2	H_20	20 †	2
Acid	A1	1 M HCl	2	4
	A2	1 M NH_4OH made 0.5 M in NH_4Cl	4	4
	A3	1 M NH_4Cl	1	4
	A4	H_2O	20 †	2

* Or until absorbed substances are eluted.

† Or until effluent is chloride-free. This will occur sooner at a faster flow rate, but additional water will be needed.

5.1 Non-Ideal Behavior

In addition to the paired retardation groups, each AG 11 A8 resin bead contains a few unpaired cation exchange groups and a few unpaired anion exchange groups. These free groups are in the ammonium and chloride forms, respectively, when the resin is shipped.

The form of these groups depends upon their past environment. Thus, chloride is used during and after

neutralization to prevent the free anion exchange groups from going into the hydroxide form. The hydroxide form is weakly held, and therefore exchanges readily to yield an undesirably high anion capacity in addition to making the resin basic.

Section 6
Applications

6.1 Adsorption of Mineral Acids

AG 11 A8 resin has the ability to absorb mineral acids while allowing the salts and organic molecules to pass through the column.[1] The strong acid absorption properties of AG 11 A8 resin make it ideal for the removal of HCI from protein hydrolysates and amino acids. The resin has an acid absorption of about 1.2 meq/ml resin. The experimental conditions are as follows:

Column	10 g AG 11 A8 resin, 50-100 mesh, 1 cm^2 x 14 cm bed
Load	13 ml (93% bed volume) amino acid mixture: glycine, glutamic acid, and arginine at 2.5 mM; HCI at 1 M
Flow rate	2 cm/min
Eluant	Water

Figure 2 shows this procedure schematically. To remove the absorbed acid (regenerate the resin), neutralize with base in the batch method, return the resin to the column, and elute with deionized water.

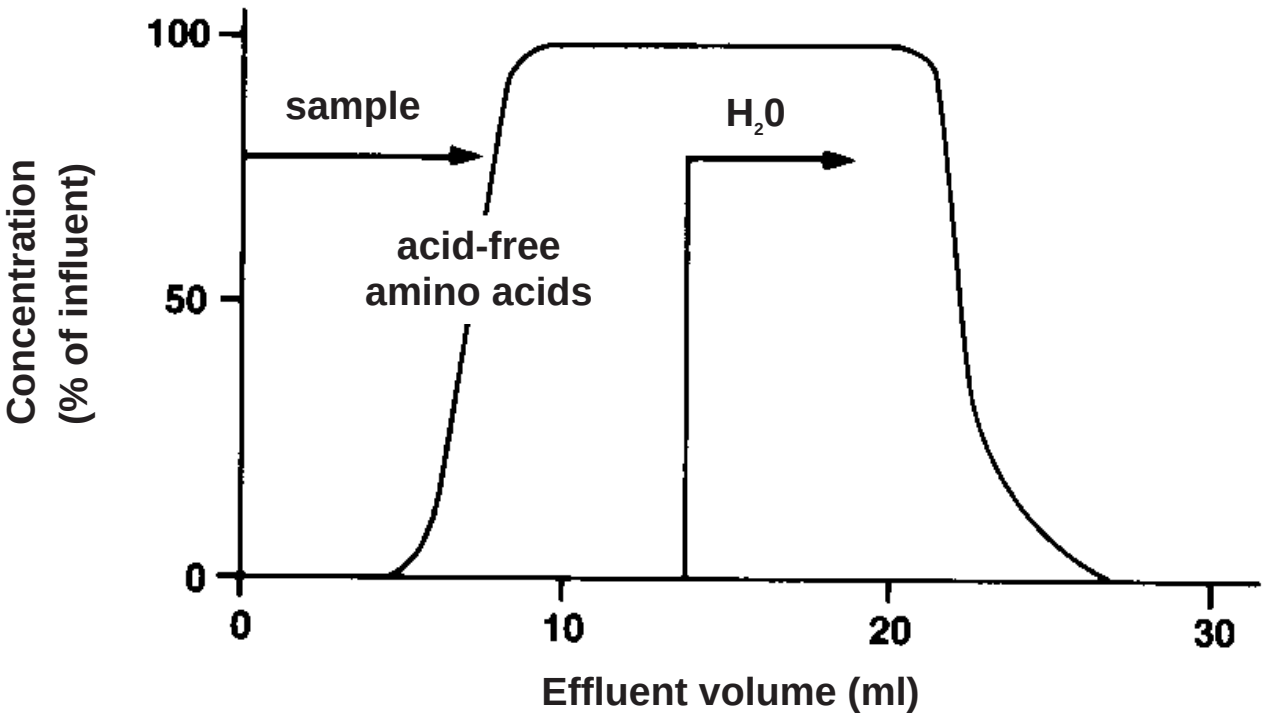

Fig. 2. Separation of HCl from amino acids.

6.2 Metal Separations

AG 11 A8 resin can be used for the separation of indium, gallium, and thallium[2] as illustrated in Figure 3. The experimental conditions were:

Column	AG 11 A8 resin, 50-100 mesh, 2.50 x 0.306 cm^2
Temperature	35° C
Flow rate	2ml/min

In addition to this separation, platinum has been separated from palladium on AG 11 A8 resin, demonstrating the potential usefulness of the resin for radiochemical separations.

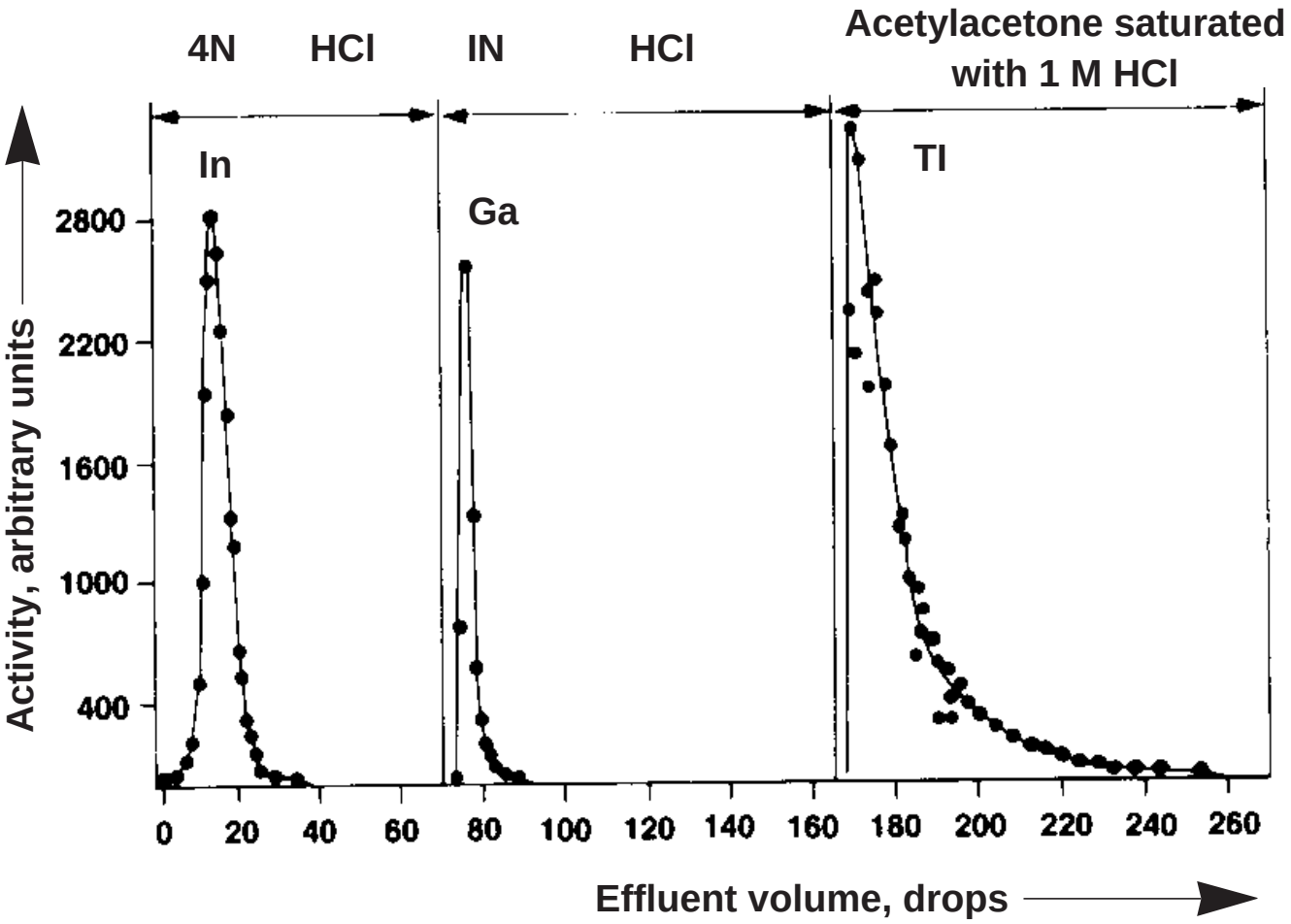

Fig. 3. Separation of In^{3+}, Ga^{3+}, Tl^{3+}.

6.3 Detergent Removal

AG 11 A8 resin provides a more rapid and efficient method for removing SDS from proteins than equilibrium dialysis, electrodialysis, or other ion exchange resin methods.[3] Kapp and Vinogradov achieved an average protein recovery of 83% and a reduction of SDS from an average of 640 moles per mole protein to an average of 0.67 moles when they used AG 11 A8 resin to remove SDS in water or sodium phosphate buffer. AG 11 A8 resin is generally effective for separating small ions from large organic compounds, such as proteins and amino acids. Under appropriate buffer conditions, it attracts SDS strongly, but absorbs very little protein. Kapp and Vinogradov found that SDS could be removed most effectively if the sample was in water or phosphate buffer. Certain buffers, such as 0.1 M Tris HCI, pH 7.0, and 0.1 M sodium borate, pH 9.0, prevented SDS removal. For samples in other buffers, good results could be achieved if the sample was desalted by gel filtration before removing the SDS. For example, Table 4 shows the results obtained with bovine serum albumin. The table shows that the results of the two-step procedure were better than for the samples initially in water, because the gel filtration step removed free SDS.

Table 4. Results of SDS Removal from BSA

Eluant	Gel Filtration Before SDS Removal	% Protein Recovery	Mole Ratio Protein: SDS Before Column	Mole Ratio Protein: SDS After Column
Water	No	90	1:220	1:1.1
0.1 M Na phosphate buffer, pH 7.2	No	92	1:560	1:1.1
0.1 M Tris	No	86	1:560	1:30
HCI buffer, pH 7.0	Yes	97	1:330	1:0.66

The gel filtration and SDS removal steps were performed in the same column, by packing the column with 10 ml gel filtration media above the AG 11 A8 resin. However, a separate gel filtration desalting step, performed as described in Table 5, also works well.

Table 5. Gel Filtration Desalting

Packing	Bio-Gel® P-6DG gel
Equilibration and elution buffer	DI water or desired new buffer
Column volume	≥ 4 x sample volume
Column height/width ratio	10:1
Flow rate	> 30 cm/min

6.4 Other Applications

Some general applications of AG 11 A8 resin are listed in Table 6.

Table 6. Applications of AG 11 A8 Resin

Application	Reference
Removal of 6M HCl from [18F]2-deoxy-2-fluoro-D-mannose	Luxen, A., Satyamurthy, N., Bida, G.T., Barrio, J.R., *Int. J. Rad. Appl. Instrum.*, **37,** 409-413 (1986).
Removal of SDS from fimbrial protein in 6M urea	Young, D.H., Stemmer, W.P., Sequeira, L., *Appl. Environ. Microbiol.*, **50,** 605-610 (1985).
Removal of 6M HCl from 2-deoxy-2[18F]fluoro-D-glucose	Adam, M.J., Ruth, T.J., Jivan, S., Pate, B.D., *Int. J. Appl. Radiat. Isot.*, **35,** 985-986 (1984).

Section 7
Storage

AG 11 A8 ion retardation resin is stable for 5 years when stored at room temperature in the original unopened container. Avoid exposure to ultraviolet light.

Section 8
Stability

AG 11 A8 ion retardation resin is stable in acid, base, and organic solvents; it is also autoclavable. Avoid exposure to strong oxidizing agents.

Section 9
References

1. Heathcote, J. G., Davies, D. M. and Haworth, C., *Clin. Chim. Acta*, **32,** 457 (1971).
2. Dybczynski, R., Use of Amphoteric Ion Exchange Retardation Resin AG 11-A8, Third Symposium on Ion Exchange.
3. Kapp, O. H. and Vinogradov, S. N., *Anal. Biochem.*, **91,** 230 (1978).
4. Jelkmann, W. and Baure, C., *Anal. Biochem.*, **75,** 382 (1976).

Section 10
Product Information

Catalog Number	Description	Mesh	Pkg. Size	Diameter (microns)	Density g/ml
142-7834	**Analytical Grade AG 11 A8 Resin**	50-100	500 g	150-300	0.70
143-7834	**Biotechnology Grade AG 11 A8 Resin**	50-100	100 g	150-300	0.70

Section 11
Technical Information

For additional information and technical assistance, contact your local Bio-Rad representative or in the US call Technical Service at 1-800-4BIORAD.

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