Toyopearl®- AF Amino-650M

TOYOPEARL
PRODUCT OVERVIEW

Introduction

Toyopearl AF-Amino-650M is a resin functionalized with a chemically reactive amine group that can be used to couple ligands bearing an aldehyde group or an activated carboxylate group. The amine functional group density (*ca 100µ*mol/ml) is ideal for the coupling of proteins or low molecular weight ligands by two methods: reductive alkylation or peptide bond formation under acidic conditions, pH 4.0 - 6.0. For example, lactose was coupled by reductive alkylation to yield a ligand density of ca 30µmol/ml resin[1]. The resin is provided slurried in water containing 0.02% sodium azide as a preservative.

Product Highlights

Toyopearl AF-Amino-650M resin is designed with physiochemical characteristics suitable for production scale chromatography:

- Hydrophilic, dimensionally stable matrix with excellent pressure/flow characteristics
- Large 1000 angstrom pores to accommodate the largest proteins
- Chemical stability to strong acid, strong alkali, and organic solvents allows severe ligand coupling conditions, harsh cleaning procedures (within the limitations of the coupled ligand) and a broad range of elution conditions
- Changes in the pH or salt concentration of the eluent does not affect the resin bed volume

Resin Type	Toyopearl AF Amino-650M
Particle Size Range	40-90µ wet resin
Functional Group Density	*ca.* 100 µmol/ml wet resin
Exclusion Limit	globular proteins: 5,000,000 Daltons, PEG: 1,000,000 +I- 30% MW
Packing Procedure	Pack in the highest salt concentration expected for the separation cycle. For a 1cm x 5cm column, use a packing velocity of 800-1000cm/hr, or maintain a pressure of 30psi. Operate the column at 30 to 130cm/hr.
Cleaning Conditions	1M NaCl, 6M urea or guanidine HCl, then starting buffer, for severe contamination: 0.5N NaOH or HCl, then H_20
Storage Conditions	H_20 with bacteriostat: 0.02% azide, 20% alcohol, thimerosol, etc. 4°C - 10°C

Applications

Carboxylic acid coupling procedure: Proteins by Carbodiimide Coupling Method

1) Quickly wash the resin on a fritted glass funnel with distilled H_20 and then with 0.5M NaCl aqueous solution adjusted to pH 4.5 - 5.0. (Use purified H_20. Buffers containing amino, carboxyl, or phosphoric acid groups that react with EDC are not recommended.)
2) Weigh out 2g (wet weight) of the washed resin.
3) Dissolve 40 - 50 mg of protein in 0.5M NaCl aqueous solution and add the solution to the resin.
4) Add 60mg of EDC [N-ethyl-N'-(3-dimethyl aminopropyl) carbodiimide hydrochloride] to the resin suspension and then agitate gently for 24 to 48 hours at 25°C. (Do not use a magnetic stirrer.)
5) Wash the resin with H_20, I M NaCl, H_20.
6) Protein densities of several mg to 25mg/1g (wet weight) of the resin are possible. Examples of coupling efficiencies are listed in the table: Carbodiimide coupling of proteins to Toyopearl AF-Amino-650M.

Carbodiimide coupling of proteins to Toyopearl AF-Amino 650M

Protein	pI	Efficiency (%)
Ovalbumin	4.2	36
Soybean trypsln Inhibitor	4.3 - 4.6	45
Bovine serum albumin	4.9	94
Human IgG	5.3 - 7.0	31
Cytochrome C	10.1	18
Lysozyme	11 - 11.4	30

Carboxylic acid coupling procedure: Oxalic Acid by Carbodiimide Coupling Method[2]

1) Quickly wash the resin on a fritted glass funnel with distilled H_2O adjusted to pH 4.7.
2) Weigh out 10g (wet weight) of the washed resin.
3) Dissolve 6g of potassium oxalate in H_2O, adjust pH to 4.7 and volume to 25ml. Add this ligand solution to the resin.
4) Add 1g of EDC and then agitate gently for 24 hours at 25°C. (Do not use a magnetic stirrer.)
5) Wash the resin thoroughly with H_2O, 1M NaCl, and finally H_2O to remove excess ligand.
6) Acetylate[3] the residual amino groups by adding 8ml of 0.2M sodium acetate and 4ml of acetk anhydride to the resin at 0°C for 30 minutes. Add another 4ml of acetic anhydride and incubate at 25°C for 30 minutes.
7) Wash the resin with H_2O, 1M NaCl, H_2O.
8) Expected oxalic acid ligand density is 2.5 µmol/l ml of the resin (determine by titration).

Aldehyde coupling procedure: Lactose by ReducUve Amination Method[1]

1) Quickly wash the resin on a fritted glass funnel with distilled H_2O, and weigh out 1g (wet weight) of resin.
2) Dissolve 0.3g of lactose in 2ml of warmed 0.2M K_2HPO_4 solution. Add this ligand solution to the resin.
3) Add 0.2g of $NaCNBH_3$ powder, and agitate gently overnight at 60°C. (Do not use a magnetic stirrer.)
4) Remove excess ligands by washing with H_2O, and then wash the resin thoroughly with 0.1M boric acid buffer, pH 8.2.
5) Add a small quantity of sodium hypochlorite to decompose the cyanic content of the solution.
6) Acetylate the residual amino groups (as described above).
7) Expected lactose ligand density is *ca.* 100 µlmol/1g of dry resin (determine by quantitative analysis for galactose liberated by acid-catalyzed hydrolysis of the resin).

Carboxylic Acid coupling procedure: Heparin by Carbodiimide Coupling Method

1) Quickly wash the resin on a fritted glass funnel with distilled H_2O adjusted to pH 4 - 6.
2) Weigh out 2g (wet weight) of the washed resin.
3) Dissolve 150mg of heparin in distilled H_2O, adjust the solution to pH 4 - 6 with 0.1M HCl, and make up 1ml.
4) Add this ligand solution to the resin, and agitate gently overnight at 4°C. (Do not use a magnetic stirrer.)
5) Add 1ml of EDC (30 mg/ml) and adjust to pH 4 - 6 with 0.1M HCl. Repeat two more times, agitate overnight at 4°C.
6) Wash with H_2O, 0.1 M $NaHCO_3$ with 0.5 NaCl, H_2O, 0.5M acetic acid buffer at pH 4, and finally H_2O.
7) Acetylate the residual amino groups (as described above).
8) Expected heparin ligand density is *ca.* 20 mg/l 9 of dry resin (determine by quantitative analysis for sulfur).

Aldehyde coupling procedure: Heparin by Reductive Amination Method[4]

1) Quickly wash resin on a fritted glass funnel with distilled H_2O and then with 0.2M K_2HPO_4.
2) Weigh out 2g (wet weight) of the washed resin.
3) Dissolve 60ml of heparin in 2ml of 0.2M K_2HPO_4 and add this ligand solution to the resin.
4) Add 20mg of $NaCNBH_3$ and agitate gently overnight at 60°C. (Do not use a magnetic stirrer.)
5) Wash with H_2O, 0.1M $NaHCO_3$ with 0.5 NaCl, H_2O, 0.5M acetic acid buffer at pH 4, and finally H_2O.
6) Add a small quantity of sodium hypochlorite to decompose the cyanic content of the solution.
7) Acetylate the residual amino groups (as described above).
8) Expected heparin ligand density is *ca*. 8 mg/l 9 of dry resin (determine by quantitative analysis for sulfur).

References:
(1) Malsumolo, I.; Ito, Y.; and Seno, N.: J. Chromatogr., 239, 747 754 (1982).
(2) O'Carra, P. and Barry, S.: FEBS Letters, 21, 281 (1972).
(3) Baues,R..l.and Gray, G.R.: J.Bid.Chem.,242,57(1977),and Matsumoto,l.et al: Anal. Biohem.,116,103(1981).
(4) Fukunashi, M. et al: Anal. Blochem., 126, 414 (1982).

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