Toyopearl®- AffiPak™ ACT LabPak Sampler

TOYOPEARL
PRODUCT OVERVIEW

Introduction

Toyopearl AFFIPAK ACT LABPAK Sampler contains two resins functionalized with chemically active groups ready for the direct coupling of a protein or other ligand. Toyopearl AF-Tresyl-650 and Toyopearl AF-Epoxy-650M are pre-activated resins that are highly reactive toward amine and thiol groups. The former is recommended for the coupling of proteins, while the latter is recommended for introducing high densities of low molecular weight ligands. Both resins are provided in a freeze dried state.

Product Highlights

Toyopearl Affinity resins are 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

Typical Properties of Toyopearl Activated Affinity Resins

Resin Type	AF Epoxy 650M	AF Tresyl-650M
Particle Size Range	40-90µ wet resin	40-90µ wet resin
Functional Group Density	80 µmol/ml wet resin	80 µmol/ml wet resin
Coupled Protein Capacity	NA	>60mg/g dry resin (soybean trypsin inhibitor)
Exclusion Limit	globular proteins: 5,000,000 Daltons, PEG: 1,000,000 +I- 30% MW	
Hydration Procedure	Swell in H_20, wash 3x, 19 = *ca* 3.5-4ml wet resin	
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 Store dry, unreacted resin below 0°C	

Typical Protein Coupling Capabilities of AF-Tresyl-650M

Protein	?
Soybean trypsIn Inhibitor	16mg/mL wet resin
Human IgG	10mg/mL wet resin
Lysozyme	60mg/mL wet resin
Bovine serum albumin	12mg/mL wet resin
α-Chymotrypsin	12mg/mL wet resin
Myoglobin	12mg/mL wet resin

Applications: Toyopearl AF-Epoxy-650M

The high density of epoxy functionality (ca 200-250 µmol/ml) is especially useful for generating specialized affinity supports with low molecular weight ligands. For example, glutathione and glycine have coupled at densities >100 µmol/ml hydrated,)resin, as described below. For special applications, Toyopearl AF-Epoxy-650M may be converted to hydrazide[1] or diazonium salt[2] bearing supports. The former are useful in the immobilization of carbohydrates or glycoproteins, the latter for proteins with accessible tyrosine or histidine residues. May be used from pH 2-12.

Amine coupling procedure: Glycine

1) Quickly wash the rehydrated resin on a fritted glass funnel with H_2O. Weigh out 5g.
2) Dissolve 1.5g of glycine in 2N NaOH solution, adjust to pH 11, and make *ca* 10ml.
3) Add the glycine solution to the resin and aggregate gently for at least 8 hours at 45°C. (Do not use a magnetic stirrer.)
4) Wash the resin sequentially with H_2O,1M NaCl, and H_2O to remove excess glycine.
5) Add 1M ethanol-amine to the resin and agitate gently overnight to block excess active groups.
6) Expected glycine ligand capacity is 100 µlmol/ml wet resin (determine by titration).

Thiol coupling procedure: Glutathione

1) Weigh out 0.5g of the wet resin, wash with 0.1M phosphate buffer, pH 7, and then add 4ml of buffer to the resin.
2) Dissolve 100mg of glutathione in H_2O, adjust to pH 7 with aq. KOH, and make *ca* 1.0ml.
3) Add the glutathione solution to the resin and agitate gently for 24 hours at 37°C. (Do not use a magnetic stirrer.)
4) Wash the resin sequentially with H_2O,1M NaCl, and H_2O to remove excess glutathione.
5) Add 1M ethanol-amine to the resin and shake overnight to block excess active groups.
6) Expected glutathione ligand capacity is 100 µmol/1g wet resin (determine by quantitating the unreacted glutathione).

Applications: Toyopearl AF-Tresyl-650M

The moderate density of tresyl functionality (*ca* 20 µmol/ml hydrated resin) allows substantial protein binding without excessive multi-point attachment which may impair ligand activity. A typical coupling may be accomplished in 1M potassium phosphate buffer, pH 7-8 at 4°C to 25°C. This resin is ideal for coupling proteins under mild conditions.

Protein A coupling procedure[3]

1) Dissolve 2.5mg of recombinant Protein A in 4ml of 0.05M Tris-HCl, pH 8, containing 0.5M NaCl.
2) Weigh out 0.4g of the dry resin and add to the Protein A solution.
3) Agitate gently for 2 hours at 25°C and then wash 3 x 20ml with starting buffer. (Do not use a magnetic stirrer.)
4) Add 10ml of 0.1M Tris-HCl, pH 8.5, and agitate gently for 1 hour to block excess active groups.
5) Expected Protein A ligand capacity is 0.6 mg/ml wet resin (determine by amino acid analysis).
6) Expected IgG binding capacity is 4.6 mg/ml wet resin, with quantitative recovery. The competing nucleophilic buffer in this unique coupling procedure limits the multipoint attachment of the Protein A. Incomplete elution of bound bG is often attributed to Protein A immobilized with too many linkages.

Concanavalin A coupling procedure[3]

1) Dissolve 15mg of Con A from Jack bean in 4ml of 0.1 M carbonate buffer, pH 8, containing 0.5M NaCl.
2) Weigh out 0.4g of the dry resin and add to the Con A solution.
3) Agitate gently for 4 hours at 25°C and then wash 3 x 20ml with starting buffer. (Do not use a magnetic stirrer.)
4) Add 10ml of 0.1M Tris-HCl, pH 8.5, and shake for 1 hour to block excess active groups.
5) Expected Con A ligand capacity is 12 mg/ml wet resin, (determine by amino acid analysis).
6) Expected peroxidase binding capacity is 4.8 mg/ml wet resin, and the bound fraction contains 70% of the activity.

References:

(1) "Preparation of High Capacity Adsorbents for Lectins Using New Hydrazino-Carriers," I. Matsumo~o et al., Proc. of 7th International Symposium on Glycoconjugates, Lun-Ronneby, Sweden 17-23 July 1983.
(2) "Immobilization of Proteins on Toyopearl Gels," N. Hagi, S. Nishimura and K. Oyama, Toyo Soda Kenkyuhokoku, 25 Vol.2 (1981) 13.
(3) "Preparation of Adsorbents for Affinity Chromatography using TSKGEL Tresyl-Toyopearl 650M," K. Nakamura et al., Journal of Chromatography, 478 (1989) 159-167.

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