**Introduction**

Toyopearl AFFIPAK 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

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### Typical Properties of Toyopearl Activated Affinity Resins

<table>
<thead>
<tr>
<th>Resin Type</th>
<th>AF Epoxy 650M</th>
<th>AF Tresyl-650M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Size Range</td>
<td>40-90μ wet resin</td>
<td>40-90μ wet resin</td>
</tr>
<tr>
<td>Functional Group Density</td>
<td>80 μmol/ml wet resin</td>
<td>80 μmol/ml wet resin</td>
</tr>
<tr>
<td>Coupled Protein Capacity</td>
<td>NA</td>
<td>&gt;60mg/g dry resin (soybean trypsin inhibitor)</td>
</tr>
<tr>
<td>Exclusion Limit</td>
<td>globular proteins: 5,000,000 Daltons, PEG: 1,000,000 +I- 30% MW</td>
<td></td>
</tr>
<tr>
<td>Hydration Procedure</td>
<td>Swell in H₂O, wash 3x, 19 = ca 3.5-4ml wet resin</td>
<td></td>
</tr>
<tr>
<td>Packing Procedure</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Cleaning Conditions</td>
<td>1M NaCl, 6M urea or guanidine HCl, then starting buffer, for severe contamination: 0.5N NaOH or HCl, then H₂O</td>
<td></td>
</tr>
<tr>
<td>Storage Conditions</td>
<td>H₂O with bacteriostat: 0.02% azide, 20% alcohol, thimerosol, etc. 4°C - 10°C Store dry, unreacted resin below 0°C</td>
<td></td>
</tr>
</tbody>
</table>

### Typical Protein Coupling Capabilities of AF-Tresyl-650M

<table>
<thead>
<tr>
<th>Protein</th>
<th>Coupling Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean trypsin Inhibitor</td>
<td>16mg/mL wet resin</td>
</tr>
<tr>
<td>Human IgG</td>
<td>10mg/mL wet resin</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>60mg/mL wet resin</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>12mg/mL wet resin</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>12mg/mL wet resin</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>12mg/mL wet resin</td>
</tr>
</tbody>
</table>

**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 hydrazide1 or diazonium salt2 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 H₂O. 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 H₂O, 1M NaCl, and H₂O 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 μmol/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 H₂O, 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 H₂O, 1M NaCl, and H₂O 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**

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 IgG is often attributed to Protein A immobilized with too many linkages.

**Concanavalin A coupling procedure**

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.