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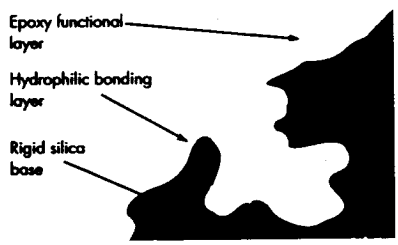
Waters New Epoxy-Activated Affinity Products: Rapid Purification of Biomolecules

A technique for the purification of biomolecules based on bioselective interactions, affinity chromatography typically results in high purification and recovery of biological activity.

Purification of Small and Large Biomolecules

Waters Protein-Pak™ Epoxy-Activated Affinity packings have a rigid non-compressible 37-55 µm silica base with a 500Å pore size. A polymerization process encapsulates the silica base with a hydrophilic bonding layer. Protein-Pak affinity is prepared by first reacting large-pore silica to form a diol-silica. The porous silica base gives the packing high available capacity for large biopolymers while the rigid structure allows high flow rates without the compression typically associated with conventional agarose based gels. Active sites of the silica are blocked by the hydrophilic bonding layer to produce low non-specific binding properties. (Figure 1)

Figure 1: Waters Protein-Pak Epoxy-Activated Support



A polymerization process encapsulates all active sites of the rigid wide-pore silica base to produce low non-specific binding properties.

Waters Protein-Pak affinity matrix contains a glycidoxypropyl moiety that results in a seven atom spacer arm. (Figure 2) The spacer arm allows the binding sites to be more accessible to the ligand which allows for high binding capacity of sample. The epoxy-activated groups covalently bond small and large ligands (Table 1) via amino, hydroxyl or sulfhydryl groups resulting in a stable linkage which is less likely to be susceptible to hydrolysis and ion-specific interactions.

There have been many ligands that have been successfully bound to the Waters Protein-Pak Epoxy-Activated material and the resulting packings have been used to purify many different biomolecules. For example, recombinant proteins A and G have been used for the purification of human and mouse IgG. Also, N-acetyl-D-glucosamine was immobilized and used for the purification of wheat germ hemagglutinin.

Figure 2: Chemistry Of Epoxy-Activated Products

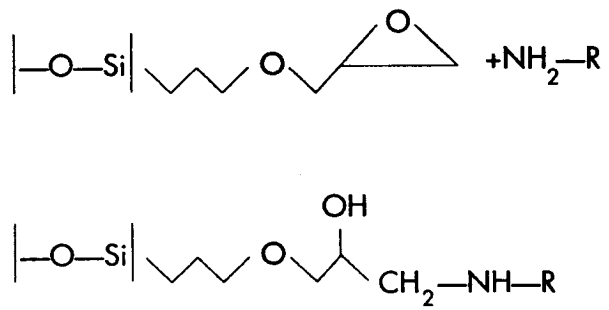
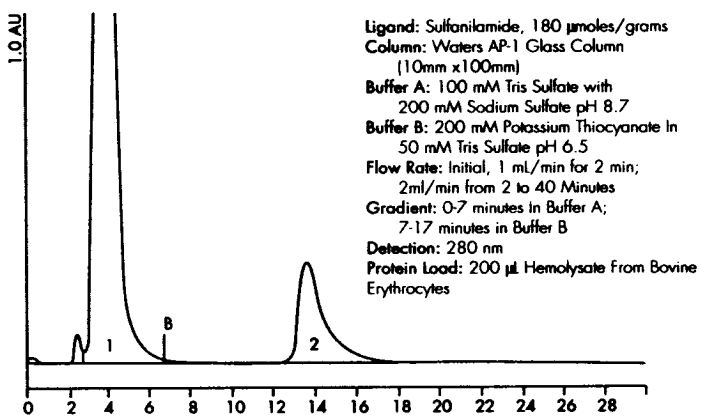


Figure 3: Protein-Pak™ Epoxy-Activated Affinity Products: Carbonic Anhydrase Purification



Biospecific Purification of Microgram to Gram Quantities

Protein-Pak affinity is supplied both in microcolumns and as bulk packing material. Protein-Pak microcolumns are perfect for scouting coupling conditions or for use with very small quantities of sample. Bulk affinity packings from Waters can be packed in any column size, such as Waters Advanced Purification (AP) Glass Columns to meet purification needs at

any scale. Also, preparative scale runs can be performed at rapid flow rates keeping run times significantly shorter than those for soft gels.

Fast Purification With High Recovery of Biological Activity

Carbonic anhydrase was purified using Protein-Pak Epoxy-Activated affinity material packed in a Waters AP Glass Column. This enzyme is an esterase found in erythrocytes that helps control the transport of molecular CO_2 through the bloodstream. Sulfanilamide, an inhibitor of carbonic anhydrase, was reacted with the epoxy-activated material at 45°C for 25 hours.

A bovine hemolysate (200µl of 24mg/ml solution) was applied to the column equilibrated with Tris sulfate at pH 8.7 (Figure 3) After washing out the unretained protein (peak 1) the buffer was switched to Tris sulfate pH 6.5 containing potassium thiocyanate to elute the carbonic anhydrase (peak 2). Esterase activity in peak 2 was measured by the hydrolysis of nitrophenyl-acetate to nitrophenol. Carbonic anhydrase was purified 8 fold with a 100% recovery of biological activity (Table 2) in less than 16 minutes.

To learn more about Waters Protein-Pak Epoxy-Activated Affinity products, check box 6 on the reply card.

Table 1: Protein-Pak Epoxy-Activated Affinity Applications

Immobilized Ligands	Applications
Gammabind-G™ and rProtein A	Human IgG Mouse IgG In Serum
Sulfanilamide	Carbonic Anhydrase
N-Acetyl-D-Glucosamine	Wheat Germ Lectin
Heparin	Lysozyme; Antithrombin III
Concanavalin A	Ribonuclease B, Horseradish Peroxidase
Cibacron Blue	Bovine Serum Albumin
Aminophenylboronic Acid	Uridine, Adenosine, Sorbital

Gammabind-G is a trademark of Genex Corp.

Table 2: Purification of Carbonic Anhydrase from Hemolysate

	Protein Concentration	Total Volume	Total Protein	Total Biological Activity	Specific Activity	Purification Factor	% Recovery of Biological Activity
Crude	23.8 mg/ml	1ml	23.8mg	8.75 Units	0.37 U/mg	1	100%
Affinity Purified Peak II	.06 mg/ml	10ml	0.59 mg	1.75 Units	2.96 U/mg	8	100%

The 200 µl of bovine hemolysate (24 mg protein/ml) applied to the column.
*Carbonic anhydrase activity was measured by converting nitrophenyl acetate to nitrophenol at pH 7.5.

Ordering Information

Waters Protein-Pak™ Epoxy-Activated Affinity Products

	Part No.	Price
25 gram bottle (50mls)	30653	$195.00
100 gram bottle (200mls)	30654	550.00
Protein-Pak Affinity microcolumn™ (10/pkg)	35955	$ 49.00

For immediate delivery call 1-800-252-HPLC.