

**Affinity Chromatography Pre-packed Columns**

Store at 2-8 °C

**Pre-Packed Columns**

Name	Product Code	Potential usage
p-Aminobenzamidine Agarose	PAB-5	Trypsin purification or removal
Heparin Agarose Type I	HEP-I-5	Enzymes (reserve transcriptase, lipases, lipoproteins)
Heparin Agarose Type II	HEP-II-5	Blood coagulation proteins, fibronectin
Hydrophobic Agarose	MAA-8	proteins, enzymes, glycoproteins
Iminodiacetic Acid Agarose	IDA-5 IDA-10	Serum proteins, collagenase, lactoferrin, Interferon

**Product Description**

Affinity chromatography is a powerful tool for protein purification. Pre-packed columns are offered as a convenience to facilitate rapid and consistent results. Column performance is determined by a number of factors. There is no general protocol for all protein purification. Choice of an affinity resin and chromatographic conditions requires essential knowledge of the specific properties of the target protein:

- A) pH profile
- B) ability to withstand low ionic strengths
- C) required stabilizers (mercaptoethanol, EDTA, DTT, and metal ions)
- D) temperature stability (resin binding capacity often increases at room temperature, but higher temperature may denature the target protein).

Column size: 2.5 ml bed volume.

**Storage/Stability**

Store the pre-packed columns at 2-8 °C in an upright position with both caps in place. Thimerosal (0.01 to 0.02%) may be added for long term storage.

**DO NOT FREEZE!**

# Product Information

**Procedure**

The following are general guidelines and suggestions for performing affinity chromatography. Conditions must be optimized for each individual target protein, based on the physical properties of that protein.

Equilibration (starting) buffer: 0.01 M Tris-HCl, pH 7.5 to 8.0. (Other buffer systems may be used if the target protein is unstable in Tris buffer).

Additional buffer components may be essential for protein stability, (mercaptoethanol, EDTA, and divalent metal ions [Zn<sup>+2</sup>, Mg<sup>+2</sup>, Ca<sup>+2</sup>]).

Elution buffer: 0.01 M Tris-HCl, pH 7.5 to 8.0 with 1.5 M NaCl. Alternative salts may be used [KCl, CaCl<sub>2</sub>, NH<sub>4</sub>Cl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>].

Specific eluants (5 to 50 mM): nucleotides, cofactors, coenzymes, and substrates

Chaotropic agents: (0.5 to 6 M) urea, guanidine, sodium thiocyanate.

Triton X-100 (0.1 to 2%) or ethylene glycol (0.1 to 2%)

pH shifts (use with care): pH range 3.2 to 10

**Sample preparation:**

- A) Centrifugation eliminates particulates and minimizes lipid or lipoprotein content (this will aid in resin cleaning and extend column life).
- B) Sample concentration should be between 1 to 10 mg of protein per ml of sample
- C) Equilibrate sample in starting buffer: (dialysis, desalting columns, diafiltration or dilution)

Chromatographic Conditions: Suggested operating temperature is 2-8 °C.

- A) Equilibrate each column with 5 to 10 column volumes of the starting buffer for the target protein.
- B) Load the protein sample onto the column.
- C) Wash the sample into the column with a small volume (0.1 to 0.5 ml) of the equilibration buffer.
- D) Continue washing with the equilibration buffer to remove unbound protein. Washing may require 3 to 10 column volumes for complete removal of unbound protein.
- E) Elute bound protein with the appropriate elution buffer.
- F) Assay elution fractions for the target protein.

G) The column may be regenerated with the following procedure. Wash the column with 10 column volumes of each:

- 1) 0.1 M borate buffer, pH 9.8 + 1.0 M NaCl
- 2) 0.1 M borate buffer, pH 9.8
- 3) Deionized water or distilled water.
- 4) 2.0 M NaCl
- 5) Equilibration buffer

## Results

Variables that will effect protein binding capacities:

- A) Lack of consistency in protein samples
  - 1) partial degradation or denaturation of the target protein
  - 2) different contaminating proteins present in the protein sample that may inhibit binding or compete for binding sites
- B) Resin not properly equilibrated (ionic strength incompatible to protein binding)
- C) Inconsistencies in elution techniques
- D) Condition of the resin (age and effectiveness of regeneration)

Chromatography Kit Guidelines	General Conditions	Specific Conditions
<b>Equilibration buffers</b>	low ionic strengths pH ranges 7-9	Hydrophobic Agarose - high ionic strength (0.5 M – 2.0 M $(\text{NH}_4)_2\text{SO}_4$ or NaCl)  Iminodiacetic Acid Agarose - divalent metals ( $\text{Zn}^{+2}$ or $\text{Fe}^{+2}$ )
<b>Elution techniques</b>	competitive cofactors, substrates  ionic strength  pH shift  Chaotropic agents: urea, guanidine, NaSCN  reduced polarity: (ethylene glycerol, dioxane)	Iminodiacetic Acid Agarose - chelating agents  Hydrophobics agarose - low ionic strength
<b>Regeneration</b>	10 volumes 0.1 M borate buffer, pH 8-10 with 1.0 M NaCl	Iminodiacetic Acid Agarose - 0.1 M EDTA, followed by divalent ions

The ability of resins to be reused will depend on the effectiveness of the regeneration procedure and the stability of the ligand and matrix.

MAM 4/01

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.