Chromatography Kits

Name	Kit #	Potential Usage
p-Aminobenzamidine Agarose	PAB-5 PAB-10	Trypsin purification or removal
m-Aminophenylboronic Acid Agarose	APB-5 APB-10	Nucleotides, nucleosides, nucleic acids, enzymes (i.e., cholinesterase, 5'nucleotidase, urease.)
Aprotinin Agarose	AP-5 AP-10	Trypsin purification or removal, elastase
Heparin Agarose Type	HEP-I-5 HEP-I-10	Enzymes (reverse transcriptase, lipases, lipo-proteins)
Heparin Agarose Type II	HEP-II-5 HEP-II-10	Blood coagulation proteins, fibronectin
Hydrophobic Agaroses	MAA-8 DAA-8	proteins, enzymes glycoproteins
Iminodiacetic Acid Agarose	IDA-5 IDA-10	Serum proteins, collagenase, lactoferrin, Interferon

Affinity chromatography is the state of the art in protein purification. As a convenience to our customers, Sigma is offering pre-packed columns to facilitate rapid and consistent results. There is no ideal protocol for all protein purification. Here are some general guidelines for proper protocol development.

Proper column performance is going to be regulated by a number of factors, not the least of which is technique.

Before choosing a resin it is essential to know the specific requirements of the target protein:

A) pH requirements
B) ability to withstand low ionic strengths
C) required stabilizers (i.e., mercaptoethanol, EDTA, DTT, Metal Ions)
D) temperature (binding capacity may often be increased by room temperature operations but higher temperature may be denaturing to individual protein).

Once these questions have been answered or approximated (about your specific target proteins), chromatography can proceed.

Suggestions for use: Column size: 2.5 mls bed volume.

1) Equilibration buffer: 0.01M Tris-HCl pH 7.5 to 8.0
(Other buffer systems may be substituted if the target protein is unstable in Tris buffers). Buffer additions are acceptable and at times essential for protein stability (i.e., Mercaptoethanol, EDTA).

   Binding enhancers, such as divalent metal ions (i.e., Zn^{+2} Mg^{+2} Ca^{+2}) may also be utilized.

2) Elution buffer: 0.01M Tris-HCl pH 7.5-8.0 + 1.5 M NaCl. Alternative salts may be used (KCl, $CaCl_2$, NH_4Cl, $(NH_4)_2SO_4$)

   Specific eluants; (5 mM-50 mM) nucleotides, cofactors, coenzymes

   Chaotropic agents; (0.5 M to 6 M) Urea, guanidine, sodium thiocyanate, Triton X-100 (0.1%—2%) or ethylene glycol (0.1%-2%)

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

3) Sample preparation:

   A) Centrifugation — to eliminate particulates
   — minimize lipid or lipo-protein content (this will aid in resin cleaning and extend column life).

   B) Concentration - between 1-10 mg/ml

   C) Equilibration to column conditions
   - by dialysis
   - by desalting columns
   - by diafiltration
   — by dilution

Procedure: Recommended running temperature 3-8°C

1) Equilibrate each column used with 5-10 column volumes of the appropriate buffer for the target protein.
2) Load the protein solution on to the column.
3) Wash the load into the column with a small volume (0.1-0.5 mls) of equilibration buffer.
4) Continue washing with equilibration buffer to remove unbound protein. Washing may require 3-10 column volumes for complete removal of free protein.
5) Elute bound protein with the chosen elution buffer [Note: Some proteins may require severe conditions to elute from columns].
6) Assay elution fractions for the target protein.
7) Regenerate the column as directed below.

Regeneration:

Wash the column with 10 column volumes of each:

1) 0.1 M Borate pH 9.8 + 1.0 M NaCl
2) 0.1 M Borate pH 9.8
3) Deionized water or distilled water.
4) 2.0 M NaCl

Storage: store column upright with both caps in place at 3-8°C. 0.01 to 0.02% Thimerosal may be added for long term storage. **DO NOT FREEZE!**

Variables that will effect protein binding capacities:

1) Lack of consistency in protein solutions
   A) changes in specific activity
   B) different contaminating proteins within your load solution that may inhibit or compete for binding sites
2) Inconsistencies in equilibration of resins or buffer preparation (ionic strengths incompatible to protein binding)
3) Inconsistencies in elution techniques
4) Condition of the resin (i.e., age, effectiveness of regeneration)

Chromatography Kit Guidelines

	General conditions	Specific conditions
Equilibration buffers	low ionic strength	Hydrophobic Agarose—high ionic strength
	pH ranges 7-9	(0.5 M-2.0 M$(NH_4)_2SO_4$ or NaCl)
		Iminodiacetic Acid Agarose by divalent metals (ZN^{+2} or Fe^{+2})
Elution techniques	competitive cofactors substrates ionic strength pH shifts	m-Aminophenylboronic acid Agarose—high pH above 8.0
		Iminodiacetic Acid Agarose-chelating agents
	Chaotropic agents: (i.e., urea, guanidine, NaSCN)	Hydrophobics agarose-low ionic strength
	reduced polarity: (ethylene glycol, dioxane)	m-Aminophenyboronic Acid Agarose-competing diols, low pH
Regeneration	10 vol 0.1 M Borate pH 8-10 + 1.0 M NaCl	Iminodiacetic Acid Agarose-0.1 M EDTA followed by divalent ions

Reuse: The ability of these resins to be reused will depend on the quality of regeneration and the ability of the ligand to withstand regeneration.