Hydrophobic Chromotography Test Kits
MAA-8, DAA-8, PHE-5, PH2-5, PHE-10, PH2-10

Hydrophobic ligands can be utilized as bioselective adsorbents. Both ω-Aminoalkyl and alkyl agaroses may interact with regions of hydrophobicity inherent to most proteins.

We are providing a testing system that will be useful in designing purification protocols for individual proteins. Kit MAA-8 and DAA-8 include 8 different columns of discrete carbon lengths that will allow a researcher to quickly and easily identify which resin will most effectively purify the target protein. Kits (PHE-5 & PH2-5) containing individual hydrophobic resins are also available for protocol development.

Suggestions for use:

	Hydrophobic Conditions	Ionic Conditions
1) Equilibration buffers:	0.01 M Tris-HCl pH 7.0-8.0 + (0.5-1.5 M NaCl or 1.0 M-2.0 M Ammonium Sulphate)	0.01 M Tris-HCl pH 7.0-8.0

[Other buffers salts may be substituted if the target protein is unstable in Tris buffer. Buffer additions are acceptable and at times essential for protein stability (i.e. Mercaptoethanol, EDTA).]

2) Elution buffers:	equilibration buffer without NaCl or Ammonium Sulfate	equilibration buffer + 0.5 M —1.0 M NaCl or 1-2 M Ammonium Sulfate

[Specific eluants: High salt with the addition of hydrophobic solvents (i.e. ethylene glycol) or detergents]

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 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 the column. (i.e. 50% butanol/buffer solutions or 50% ethanol/buffer solutions)]

6) Assay elution fractions for the target protein.

7) Evaluate binding capacity vs total recovery to determine:

   A) maximum binding effectiveness for differing substitutions

   B) maximum recovery

   C) ease of recovery

   D) degree of purification

8) Regenerate the column as directed below.

Regeneration:

Wash the column with 10 column volumes of each:

1) 0.05 M NaOH
2) 0.1 M Acetate pH 4.5
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!**

Trouble Shooting:

Problem	Solution
A) irreversible binding	A) Proper choice of ligand length
B) Denaturing of target protein	B)i) Rapid post-column treatment (i.e. desalting columns, diafiltration or dialysis.) ii) Utilizing a less hydrophobic column which would generally require less denaturing elution conditions.