Hydrophobic Chromatography 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:

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<tr>
<th>Hydrophobic Conditions</th>
<th>Ionic Conditions</th>
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<tbody>
<tr>
<td>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)</td>
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[Other buffer 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:**

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