Product Information

Iminodiacetic Acid Agarose

Saline Suspension

I4758

Storage Temperature 2-8 °C

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:

- pH profile
- Ability to withstand low ionic strengths
- Required stabilizers (mercaptoethanol, EDTA, DTT, and metal ions)
- 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. Preservative may be added for long term storage. **Do Not Freeze**.

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^{+2}, Mg^{+2}, Ca^{+2}]).

Elution Buffer

0.01 M Tris-HCl, pH 7.5 to 8.0 with 1.5 M NaCl. Alternative salts may be used [KCl, $CaCl_2$, NH_4Cl, $(NH_4)_2SO_4$]. 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

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

Chromatographic Conditions

(Suggested operating temperature is 2-8 °C)

1. Equilibrate each column with 5 to 10 column volumes of the starting buffer for the target protein.
2. Load the protein sample onto the column.
3. Wash the sample into the column with a small volume (0.1 to 0.5 mL) of the equilibration buffer.
4. Continue washing with the equilibration buffer to remove unbound protein. Washing may require 3 to 10 column volumes for complete removal of unbound protein.
5. Elute bound protein with the appropriate elution buffer.
6. Assay elution fractions for the target protein.

7. The column may be regenerated with the following procedure. Wash the column with 10 column volumes of each (in order):
    - 0.1 M borate buffer, pH 9.8 + 1.0 M NaCl
    - 0.1 M borate buffer, pH 9.8
    - Deionized water or distilled water
    - 2.0 M NaCl
    - Equilibration buffer

Results

Variables that will affect protein binding capacities:

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

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	General Conditions	Specific Conditions
Equilibration buffers	Low ionic strength pH ranges 7-9	Divalent metals (Zn^{+2} or Fe^{+2})
Elution techniques	Competitive cofactors substrates Ionic Strength pH shifts Chaotropic agents: urea, guanidine, NaSCN Reduced polarity: ethylene glycol, dioxane	Chelating agents
Regeneration	10 vol 0.1 M Borate buffer, pH 8-10 with 1.0 M NaCl	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.