

Protein Purification Using Anti-DYKDDDDK (1002AH1) Affinity Gel

Materials Needed

Reagents

- Anti-DYKDDDDK (1002AH1) Affinity Gel (Cat# 700501, 700502, 700503)
- Equilibration / Binding Buffer (FLAG Buffer A): 20 mM Tris, 150 mM NaCl, pH 7.4 - 8.0
- Wash Buffer: FLAG Buffer A with 0.01–0.1% non-ionic detergent (e.g., Tween-20 or NP-40)
- Elution Buffer (Competitive Elution): FLAG Buffer A + 100–300 µg/mL FLAG peptide (DYKDDDDK)
- Alternative Elution Buffer (Low pH): 0.1 M glycine-HCl, pH 2.5–3.0
- Neutralization Buffer (for low pH elution): 1 M Tris-HCl, pH 8.5

Equipment

- Gravity flow column (e.g., chromatography column)
- Tube rotator or stir plate (for batch purification)
- Collection tubes
- UV spectrophotometer or Bradford assay reagents
- Centrifuge or filtration unit for sample clarification

Protocol

A. Resin Preparation

1. Gently resuspend the 1002AH1 resin until a uniform slurry is obtained.
 2. Transfer the desired volume of resin to an empty column.
 3. Allow the resin to settle by gravity and drain the storage buffer completely without allowing the resin to dry.
 4. Wash the resin with ≥ 3 column volumes (CV) of FLAG Buffer A to equilibrate.
- The resin is now ready for binding.

B. Binding of of DYKDDDDK-Tagged Protein**Column Method**

1. Clarify the lysate by centrifugation ($\geq 10,000 \times g$, 10–20 min) or filtration.
2. Load the sample onto the equilibrated column at a slow flow rate (0.2–0.5 mL/min) to maximize binding.
3. Collect the flow-through for analysis if desired.

Batch Method

1. Add equilibrated resin directly to the clarified lysate or supernatant.
2. Incubate with gentle mixing (rotator) at: 4°C for 2–4 hours, or overnight at 4°C for maximal binding efficiency.
3. Transfer the mixture to a column and allow the resin to settle.
4. Drain unbound material.

C. Washing

1. Wash the resin with 5–10 CV of Wash Buffer.
2. Continue washing until baseline absorbance is achieved (A280).

D. Elution**Preferred: Competitive Elution (DYKDDDDK Peptide)**

1. Add DYKDDDDK peptide (100–300 $\mu\text{g/mL}$) in FLAG Buffer A.
2. Incubate on-column for 5–10 minutes or apply slowly.
3. Collect eluted fractions.
4. Repeat elution (2–3 times) to maximize recovery.

Alternative: Low pH Elution

1. Elute with 0.1 M glycine-HCl, pH 2.5–3.0.
2. Immediately neutralize fractions with 1 M Tris-HCl, pH 8.5.

Note: Competitive elution is recommended for preserving protein activity and antibody integrity.

E. Column Cleaning After Use

1. Wash with 5 CV of FLAG Buffer A.
2. Wash with 5 CV of PBS or high-salt buffer (e.g., 500 mM NaCl).
3. For deeper cleaning, wash with 0.1 M glycine pH 2.5, followed by immediate re-equilibration.



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4. Re-equilibrate with 5–10 CV of FLAG Buffer A..

F. Resin Storage

1. Store resin at 4°C in FLAG Buffer A containing 0.02–0.05% sodium azide or 20% ethanol.
2. Do not freeze.
3. Resin can typically be reused multiple times depending on sample cleanliness and handling.