Standard Operating Procedure

Protein Purification Protocol

Protocol ID: SOP-BIO-001
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1. Purpose

This protocol describes the standard procedure for purifying recombinant proteins using affinity chromatography with nickel-NTA resin.

2. Materials Required

Item	Quantity	Supplier
Ni-NTA Agarose	5 mL	Qiagen
Imidazole	50 g	Sigma
Tris-HCl Buffer	1 L	In-house
NaCl	100 g	Fisher
Protease Inhibitor	1 tablet	Roche

3. Procedure

Step 1: Equilibrate Ni-NTA column with 10 column volumes of binding buffer (20 mM Tris-HCl, 500 mM NaCl, pH 7.4)

Step 2: Load clarified cell lysate onto the column at 1 mL/min flow rate

Step 3: Wash with 20 column volumes of binding buffer containing 20 mM imidazole

Step 4: Elute bound protein with buffer containing 250 mM imidazole

Step 5: Collect 1 mL fractions and analyze by SDS-PAGE

Step 6: Pool fractions containing target protein

Step 7: Dialyze against storage buffer overnight at 4°C

Step 8: Determine protein concentration using Bradford assay

Step 9: Aliquot and store at -80°C

4. Expected Results

Parameter	Expected Value
Protein Yield	10-50 mg/L culture
Purity	>95% by SDS-PAGE
Activity	Retained
Typical Time	4-6 hours

5. Safety Considerations

- Wear appropriate PPE including lab coat, gloves, and safety glasses
- Work in designated biosafety cabinet
- Follow chemical handling guidelines for imidazole
- Dispose of biological waste according to institutional guidelines

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