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AFFINITY PURIFICATION OF SOLUBLE HIS-TAGGED PROTEINS

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1. **Lyse cells:**

a. Suspend the cell pellet in 30–35 ml of His-washing buffer with 10 mM PMSF.
b. Sonicate cells in an ice-bath at 200 W for 6 min.
c. Rotate the lysed solution for 1 h at 4°C.
d. Centrifuge the cell lysate for approximately 13 min at 8000 rpm, 4°C.

2. **Bind protein to beads:**

a. Transfer the supernatant to 600 µl of His-beads.
b. Rotate the mixture overnight at 4°C.
c. Collect the beads by centrifugation at 2000 rpm for 10–30 seconds, 4°C. The protein-bound beads are collected in eppendorf tubes.

3. **Wash away the unbound proteins from beads:**

a. Wash the beads 3 times with 1 ml of His-washing buffer. Discard the supernatant.

4. **Elute proteins from beads:**

a. Add 300 µl of His-elution buffer to the beads.
b. Rotate the mixture for 1 h at 4°C.
c. Collect the supernatant by centrifugation at 300 rpm for 10–30 seconds.
d. Repeat steps 4 a–c.
e. Combine the eluent (total volume of 600 µl).
f. Check the molecular weight and purity of the enriched protein by SDS-PAGE analysis.

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Buffers Needed

His-washing buffer	1000 ml
20 mM Imidazole	1.36 g
1x PBS buffer	1000 ml
Adjust to pH 7.0	

His-elution buffer	1000 ml
300 mM Imidazole	20.42 g
10% Glycerol	100 ml
1x PBST buffer	900 ml
Adjust to pH 7.0	

GST-washing buffer (PBST buffer)	1000 ml
58 mM Na_2HPO_4	8.24 g
17 mM NaH_2PO_4	2.04 g
68 mM NaCl	3.98 g
Add ddH_2O to 1000 ml	
Adjust to pH 7.4	

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