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Nickel Resin Protocol for His-Tagged Protein Purification

Overview:
Nickel resin can be used to purify His-tagged recombinant proteins using either batch or column purification methods.

Resin Preparation:
Thoroughly resuspend the nickel resin by gently inverting the container until the resin is uniformly mixed. Transfer the desired volume of resin into an empty column and allow the beads to settle. Drain the storage buffer completely—**do not allow the resin to run dry.** Wash the resin with at least 2 column volumes (based on settled resin volume) of Ni-A buffer (20 mM Tris, 300 mM NaCl, pH 8.0) to remove residual ethanol. The resin is now ready for use.

Binding and Washing:

- **Column Method:** Load the clarified cell lysate or culture supernatant onto the column at a flow rate of 0.5–1.0 mL/min.
- **Batch Method:** Add washed nickel resin to the lysate or supernatant and mix gently on a stir plate or rotator at 50–70 rpm. Incubate at room temperature for 3–5 hours or overnight at 4°C for optimal binding.
- **Optional:** Add 10–40 mM imidazole to the lysate or supernatant before binding to reduce nonspecific interactions.

After binding:

- For **batch purification**, transfer the resin/sample mixture into a column (e.g., Bio-Rad Econo-Column), allow the resin to settle, and drain.
- For **column purification**, once the lysate has completely passed through the column, proceed to washing.

Wash the resin with Ni-A buffer until nonspecific proteins are removed. Monitor the flow-through using Bradford assay or UV absorbance until the signal returns to baseline.
To further reduce nonspecific binding, washing with low concentrations of imidazole (20–100 mM in Ni-A buffer) can be beneficial. A titration may be required to optimize conditions for each target protein.

Elution:

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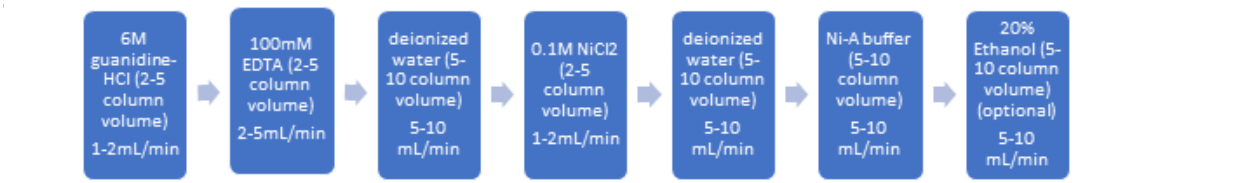
Elute the bound protein using 200–500 mM imidazole in Ni-A buffer. The optimal elution concentration should be determined experimentally for each specific target.

Cleaning After Use:
After use, wash the column with 5 column volumes of deionized water. Clean with 1 M NaOH at a flow rate of 1–2 mL/min for approximately 20 minutes. Re-equilibrate the resin with 5–10 column volumes of Ni-A buffer.

Resin Regeneration:
To regenerate the resin, use the following sequence:

1. **6 M Guanidine-HCl** (2–5 column volumes at 1–2 mL/min) – denaturing wash
2. **100 mM EDTA** (2–5 column volumes at 2–5 mL/min) – metal stripping
3. **Deionized Water** (5–10 column volumes at 5–10 mL/min)
4. **0.1 M $NiCl_2$** (2–5 column volumes at 1–2 mL/min) – resin recharging
5. **Deionized Water** (5–10 column volumes at 5–10 mL/min)
6. **Ni-A Buffer** (5–10 column volumes at 5–10 mL/min)
7. **20% Ethanol** (5–10 column volumes at 5–10 mL/min) – optional for long-term storage



Storage:
After cleaning or regeneration, transfer the resin to a suitable container and store at 4°C. Nickel resin is stable in Ni-A buffer for 1–2 months at 4°C. For long-term storage, use Ni-A buffer containing 20% ethanol and store at 4°C. The resin can typically be reused at least 3 times without significant loss of binding capacity.