Nickel purification for 6X-His tagged proteins

For our lab purposes, proteins are generally expressed using pET-vectors (Novagen). These proteins are equipped with a 6X-His tag, which adopts a conformation that is optimal for coordination to divalent nickel ions, Ni^{2+}. We use Ni-NTA superflow (Qiagen) for the resin, and the amount of this resin to the lysate containing the tagged protein should be determined empirically (see reference below).

For info on nickel-affinity purification, see "The QIAexpressionist" (www.qiagen.com)

Conduct in cold room, 4°C, or keep fractions and solutions on ice at all times to prevent protein degradation.

1. Incubate lysate containing protein of interest with nickel resin, Ni-NTA superflow, for 1—4 hours at 4°C with gentle rocking/shaking.
2. After incubation, add mixture to column and collect flow-through.
3. Wash with 10X resin volume (RV) using 0 mM imidiazole (see "Solutions" below).
4. Wash with 10X RV using 20 mM imidiazole.
5. Elute with 5X RV using 100 mM imidiazole.
6. Elute with 5X RV using 100 mM imidiazole.
7. Elute with 5X RV using 250 mM imidiazole.
8. Elute with 5X RV using 250 mM imidiazole.
9. Analyze fractions with SDS-PAGE.

Solutions: 100 mM Tris, 300 mM NaCl, X mM imidiazole (X = 0, 20, 100, 250), pH 7.4

Adjust to pH 7.4 after the addition of imidiazole.