Nickel Chelating Superflow is an immobilized metal affinity chromatography (IMAC) resin that separates proteins via their differences in affinity to Ni^{2+}. There are a number of metal-chelating resins that differ by how strongly they bind a given metal. For instance, NTA resin is a tetra-dentate chelator, leaving two coordination sites on a transition metal available to bind complexing moieties on proteins, such as Histidine repeats. TED resins are penta-dentate, leaving one less coordination site in the chelated metal for binding proteins. Thus, TED typically has less binding capacity than NTA. IDA Chelating resins are tri-dentate and typically have very high binding capacities in comparison to other chelators, but bind the metal with less affinity. While the exact mechanism is proprietary, Nickel Chelating Superflow performs more like a tetra-dentate chelator than any other commonly known IMAC chemistry. Nickel Chelating Superflow is recommended for clarified feed streams.

Most IMAC purifications require some degree of method development for optimization. The protocol below is meant as an example for His-tagged protein purification.

Protein Purification: Protocol

Reagents:

Equilibration buffer:	50mM Sodium Phosphate, 0.3M NaCl, 10mM Imidazole, pH 8.0.
Wash buffer:	50mM Sodium Phosphate, 0.3M NaCl, 20mM Imidazole, pH 8.0.
Elution buffer:	50mM Sodium Phosphate, 0.3M NaCl, 250mM Imidazole, pH 8.0.

Method for His-tagged Protein Purification:

1. Wash resin with deionized water and prepare a 1:1 slurry. Place 400µl of slurry (200µl resin) into 5ml column with frit.

2. Equilibrate resin two times with 2ml of equilibration buffer for 2 minutes while mixing.

3. Remove and discard supernatant.

4. Load 2ml of 6XHN Beta Galactosidase Lysate (if lysate does not contain Imidazole, add to final concentration of 10mM by using 3M stock solution, pH 7.0) to resin.

5. Mix the solution for 30 minutes at room temperature.

6. Collect supernatant after 30 minutes mixing (take sample of unbound fraction for SDS-PAGE analysis).

7. Wash resin four times with 2ml of wash buffer.

8. Elute two times with 500µl of elution buffer for 10 minutes while mixing.

9. Collect and combine both elution fractions. Perform SDS-PAGE analysis.

Method for Cleaning and Regeneration:

Method for Cleaning:

The 50% ethanol wash step in the regeneration procedure described below will usually suffice to clean the resin thoroughly. But our IMAC media are also tolerant to 8M Gaunidine, 6M Urea, and high salt conditions. If contact time is less than 2 hours, 0.5M NaOH may also be used to clean the resin.

Store Nickel Chelating Superflow resin as a 70% slurry with 20% Ethanol at 2–8˚C.

Nickel Chelating Superflow resin should be regenerated after each use.

Method for Regeneration:

1. Wash the column with 3 bed volumes (BV) of 50mM Tris, 1M NaCl, pH=8.5 containing 1% Triton X-100.
2. Wash the column with 3 BV of DI water.
3. Wash the column with 3 BV of 20mM Sodium Acetate, 1M NaCl, pH= 4.5 containing 1% Triton X-100.
4. Wash the column with 5 BV of DI water.
5. Wash the column with 5 BV of 50% Ethanol.
6. Wash the column with 3 BV of 20% Ethanol.
7. Wash the column with 3 BV of DI water.
8. Wash the column with 5 BV of 100mM EDTA, pH= 8.0.
9. Wash the column with 5 BV of DI water.
10. Recharge the column with 4 BV of 150mM $NiSO_4$.
11. Wash the column with 5 BV of DI water.
12. Wash the column with 3 BV of running buffer, or store in 20% Ethanol.