IDA Chelating Cellthru BigBead is an uncharged, immobilized metal affinity chromatography (IMAC) resin that separates proteins via their differences in affinity to a given chelated metal. IDA Chelating Cellthru BigBead is recommended for unclarified 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.

1. Charge IDA Chelating Cellthru BigBead with a given metal. Instructions for charging with Ni+2 are provided below and can be easily adapted for other metal ions.
2. Wash resin with purified water and pack your column with a 1:1 slurry.
3. Equilibrate resin with ten column volumes (CV) of equilibration buffer.
4. Dialyze lysate to equilibration buffer then load on to the resin.
5. Wash resin four times with five CV of wash buffer.
6. Elute with four CV of elution buffer.

Method for Cleaning:

50% ethanol is recommended for cleaning IDA Chelating Cellthru BigBead.

Store IDA Chelating Cellthru BigBead resin as a 70% slurry with 20% Ethanol at 2–8˚C.

IDA Chelating Cellthru BigBead resin should be regenerated after each use.

Method for Cleaning/Regeneration with $NiSO_4$:

Note: For initial charging follow steps 9-12

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