Denaturing Purification His-Tagged Proteins From Mammalian Cells

1. Transfect cells with His-tagged construct
2. 36 hrs later, wash cells once in ice-cold 1X PBS
3. Collect cells in ice-cold 1X PBS
4. Add sample buffer to 10% of the cells to be used as input control
5. Remaining cells spin down at 4C
6. Resuspend in 6 mL Denaturing Lysis Buffer (containing BME)
7. Add 75 uL Ni-NTA Agarose beads
8. Incubate rotating at room temperature for 4 hrs
9. Wash beads for 5 min at room temperature successively in 750 uL each:
    a. Denaturing Lysis Buffer without imidazole
    b. Buffer A pH 8.0 without Triton X-100
    c. Buffer A pH 6.3 + 0.2% Triton X-100
    d. Buffer A pH 6.3 + 0.1% Triton X-100
10. Elute proteins with 75 uL Elution Buffer at room temperature for 20 min
11. Add 4X sample buffer to eluted proteins
12. Boil samples for 5 min
13. Run Input and Eluted protein samples on SDS-PAGE
14. Western Blot with appropriate antibodies

Denaturing Lysis Buffer

6 M Guanidinium-HCl
100 mM Na_2HPO_4-NaH_2PO_4
10 mM Tris-HCl pH 8.0
5 mM Imidazole (leave out in wash buffer)
10 mM Beta-Mercaptoethanol (add fresh at time of use)

Buffer A

8 M Urea
100 mM Na_2HPO_4-NaH_2PO_4
10 mM Tris-HCl pH 8.0 or 6.3
10 mM Beta-Mercaptoethanol (add fresh at time of use)
0.2% or 0.1% Triton X-100

Elution Buffer

200 mM Imidazole
150 mM Tris-HCl pH 6.7
30% Glycerol
5% SDS
720 mM Beta-Mercaptoethanol (add fresh at time of use)