IP Protocol from Pharma Customer using Peggy (Size Mode)

1. **Cell lysis:** Lysis Buffer: 50mM Tris-HCl (pH7.5), 150 mM NaCl, 1.1% octyl glucoside.
    a. Lyse cells in buffer. Note: All cells are different in size and protein concentration. Protein concentration should be ~5 mg/mL. Clear lysate by centrifugation at 15,000rpm for 10 min @ 4°C.
    b. Estimate protein by BCA (Pierce: cat#23225)

2. **IP protocol:** Using either a biotinylated antibody (when using SA beads) or biotinylated peptide:
    a. Mix 2mg of cleared protein lysate with either 100 µM peptide or 100 µg/mL antibody, overnight at 4°C, continuous rocking.
    b. Following 24 h, prepare streptavidin magnetic beads.
        i. Briefly take 50 µL of beads Streptavidin magnetic beads (Pierce: cat#88817) and wash three times with excess PBS (1.5mL each time).
    c. Add IP complex to cleared beads. Rock for ~1hr at room temperature
    d. Using a magnetic column, isolate beads and remove lysate (retain for future use if desired). Wash three times with excess lysis buffer.

3. **Prepare samples for Peggy:**
    a. Add 65ul of sample standard in 1xMM format directly to the beads.
    b. Heat @ 95°C for 5 minutes to elute protein from beads.
    c. Clear beads by magnet.
    d. Retain 1xMM eluate solution containing IP mixture.
    e. Follow Peggy size protocol (5uL IP sample per well).