Materials Required

- Streptavidin coated magnetic beads (Pierce, NEB, etc.)
- 1.5mL microcentrifuge tubes
- Binding/Wash Buffer: SBB1/SBB2 (5X SBB1/SBB2 diluted to 1X + 0.1% Tween-20)
- Elution Buffer: SRB1 (25mM EPPS, 1M NaCl, pH 7.5)
- Alternate elution buffer: SDS-PAGE reducing sample buffer
- Biotinylated *Sia*-Lectenz (SP0502B or SP2302B or SP2602B)
- Antigen sample (clarified lysates) diluted in SBB1/SBB2
- Magnetic stand (e.g., Thermo Scientific™ DynaMag™-2 Magnet, Product No. 12321D)

Pre-washing Streptavidin Magnetic Beads

Note: To ensure homogeneity, mix the beads thoroughly before use by repeated inversion.

1. Add 50µL (0.5mg) of Streptavidin Magnetic Beads into a 1.5mL microcentrifuge tube.
2. Place the tube into the magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
3. Add 1mL of SLBBT to the tube. Invert the tube several times or vortex gently to mix. Collect the beads with a magnetic stand, then remove and discard the supernatant. Repeat 3 times total.

Note: Do not allow beads to dry. If necessary, store beads in Binding/Wash buffer before proceeding with the purification protocol.

Immunoprecipitation with downstream analysis by non-biotinylated analyte

Note: This protocol will allow for greater precipitation yields, but if your antigen sample contains multiple glycosylation sites, some precomplexed biotinylated *Sia*-Lectenz will coelute. This will show up if subsequent analysis is done using a streptavidin conjugate.

1. Pre-complexing: Combine the 500 ⌈L diluted antigen sample (the analyte) with 50µg of biotinylated *Sia*-Lectenz (the "antibody"). Incubate 2 hours or overnight at 4°C with mixing.

Note: *Dilute each sample to a minimum volume of 300-500µL with cell lysis buffer or Binding/Wash Buffer. We recommend ~50µg of antigen sample to 50µg Lectenz as a starting point.*

2. Add the antigen sample/biotinylated antibody pre-complex to a 1.5mL microcentrifuge tube containing the pre-washed magnetic beads, followed by incubation at room temperature for 30 min at room temperature or 1 hour at 4°C with mixing.
3. Collect the beads with a magnetic stand and remove and save the supernatant for analysis.
4. Add 500µL of Binding/Wash Buffer to the tube and gently mix. Collect the beads and then collect the supernatant. Repeat this wash twice.

Immunoprecipitation with downstream analysis by biotinylated analyte

Note: This protocol allows for lower yields, but removes potential Sia-Lectenz contaminants from the eluate. This is useful if using a streptavidin-conjugate in downstream analysis.

1. Combine the 50 ⌈L streptavidin coated beads and 50ug biotinylated Sia Lectenz (the "antibody") and incubate at room temperature for 30 min at room temperature or 1 hour at 4°C with mixing.

Note: *Dilute each sample to a minimum volume of 300-500µL with Binding/Wash Buffer.*

2. Place the tube into the magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
3. Add 1mL of SLBBT to the tube. Invert the tube several times or vortex gently to mix. Collect the beads with a magnetic stand, then remove and discard the supernatant. Repeat 3 times total.
4. Add the 500µL antigen sample and incubate 2 hours or overnight at 4°C with mixing.
5. Collect the beads with a magnetic stand and remove and save the supernatant for analysis.
6. Add 500µL of Binding/Wash Buffer to the tube and gently mix. Collect the beads and then collect the supernatant. Repeat this wash twice.

Elution Buffer Recovery of Antigen:

1. Add 100µL of SLEB to the tube. Incubate the tube at room temperature with mixing for 10 minutes.
2. Magnetically separate the beads and save the supernatant containing target antigen.

Note: *Elution can also be done by adding SDS sample buffer and boiling beads 10-15 minutes at 95℃.*

Downstream analysis:

Downstream analysis typically consists of SDS-PAGE followed by Coomassie blue staining and/or western blotting against the antigen of interest.