Materials

- Streptavidin coated magnetic beads (e.g., Pierce Cat #PI88816)
- 1.5 mL microcentrifuge tubes
- SiaFind™ Kit(s), Biotinylated; one or more of the following:
  - Pan-Specific Lectenz®: 10 mg/mL stock (Lectenz Bio Cat #SP0502B)
  - α2,3-Specific Lectenz®: 10 mg/mL stock (Lectenz Bio Cat #SP2302B)
  - α2,6-Specific Reagent: 1 mg/mL stock (Lectenz Bio Cat #SP2602B)
- Binding Buffer:
  - 5X SiaFind™ Binding Buffer 1 (Lectenz Bio Cat #BA0101) diluted to 1X (SBB1) in Milli-Q water for Pan-Specific and α2,3-Specific Lectenz®
  - 5X SiaFind™ Binding Buffer 2 (Lectenz Bio Cat #BA0102) diluted to 1X (SBB2) in Milli-Q water for α2,6-Specific Reagent
- Wash Buffer:
  - 5X SiaFind™ Binding Buffer 1 (Lectenz Bio Cat #BA0101) diluted to 1X + 0.1% Tween-20 (SBB1T)
  - 5X SiaFind™ Binding Buffer 2 (Lectenz Bio Cat #BA0102) diluted to 1X + 0.1% Tween-20 (SBB2T)
- Elution Buffer:
  - 5X SiaFind™ Regeneration Buffer 1 (Lectenz Bio Cat #BA0301) diluted to 1X in Milli-Q water (SRB1)
- Alternate elution buffer: SDS-PAGE reducing sample buffer
- Antigen sample (clarified lysates) diluted in SBB1 or SBB2
- Magnetic stand (e.g., DynaMag™-2 Magnet, Invitrogen Cat #12321D)

Pre-washing Streptavidin Magnetic Beads

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

1. Add 0.5 mg (50 µL) of streptavidin magnetic beads to a 1.5 mL microcentrifuge tube.
2. Place the tube in the magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
3. Add 1 mL of Wash Buffer 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 buffer before proceeding with the immunoprecipitation 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 SiaFind™ will co-elute. This will show up if subsequent analysis is done using a streptavidin conjugate.*

1. Pre-complexing: Combine the 500 µL diluted antigen sample (the analyte) with 50 µg of biotinylated SiaFind™ reagent (the “antibody”). Incubate 2 h or overnight at 4°C with mixing.

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

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

Immunoprecipitation with downstream analysis by biotinylated analyte

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

1. Combine the 50 µL streptavidin magnetic beads and 50 µg biotinylated SiaFind™ reagent (the "antibody") and incubate 30 min at room temperature or 1 h at 4°C with mixing.

Note: *Dilute each sample to a minimum volume of 300-500 µL 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 1 mL of Wash Buffer 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 500 µL antigen sample and incubate 2 h at room temperature 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 500 µL of Wash Buffer to the tube and gently mix. Collect the beads and then collect the supernatant downstream analysis by SDS-PAGE. Repeat this wash twice.

Elution Buffer Recovery of Antigen:

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

Note: *If the eluate is only to be used for SDS-PAGE analysis, the elution can also be done by adding SDS sample buffer to the beads and boiling for 10-15 min at 95°C.*

Downstream analysis:

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