Materials

- Immulon 2 HB plates (Thermo Scientific Cat #3455) or other microtiter plates
- 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
- TMB Substrate Solution (e.g., Thermo Scientific Cat #34028)
- 2 M H_2SO_4

Note: It is important to use the supplied 5X Binding Buffer for making Binding and Wash Buffers. SiaFind™ reagents are salt sensitive and PBS/TBS buffers will adversely affect signal and increase background.

Protocol

1. Prepare the following solutions:
   a. Wash Buffer:
      i. 5X SiaFind™ Binding Buffer 1 (Lectenz Bio Cat #BA0101) diluted to 1X + 0.1% Tween-20 (SBB1T)
      ii. 5X SiaFind™ Binding Buffer 2 (Lectenz Bio Cat #BA0102) diluted to 1X + 0.1% Tween-20 (SBB2T)
   b. Blocking Buffer: Wash Buffer + 5% BSA
   c. Antigen Solution: 10 µg/mL antigen in Binding Buffer
   d. Biotinylated SiaFind™ Solution(s) prepared in Blocking Buffer:
      i. Pan-Specific Lectenz®: 80 µg/mL
      ii. α2,3-Specific Lectenz®: 20 µg/mL
      iii. α2,6-Specific Reagent: 0.5 µg/mL
   e. Secondary Solution: 0.5 µg/mL streptavidin-HRP conjugate (e.g., Vector Laboratories Cat #SA-5014-1) in Blocking Buffer
2. Add 100 µL Antigen Solution (10 µg/mL in Binding Buffer) to coat wells in a 96-well Immulon 2 HB plate, cover and incubate overnight at 4°C
3. Remove the Antigen Solution
4. Wash wells 3 times with 200 µL Wash Buffer
5. Block each well with 200 µL Blocking Buffer for 1 h at room temperature
6. Wash wells 3 times with 200 µL Wash Buffer
7. Add 100 µL SiaFind™ Solution to each well and incubate for 1 h at room temperature
8. Wash wells 3 times with 200 µL Wash Buffer
9. Add 100 µL Secondary Solution to each well and incubate for 1 h at room temperature
10. Wash wells 3 times with 200 µL Wash Buffer
11. Wash wells 3 times with 200 µL Binding Buffer
12. Add 50-100 µL TMB Substrate Solution and incubate at room temperature for 2 min or until color is observed
13. Stop reaction with 50-100 µL 2 M H_2SO_4
14. Measure absorption at 450 nm on a plate reader