Materials:

- 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
- SDS-PAGE sample buffer
- Polyacrylamide gel (e.g., BioRad 4-20% Mini-PROTEAN TGX™ 10-well gels Cat #4561093)
- SDS-PAGE running buffer
- Gel apparatus
- Transfer apparatus
- Transfer stacks (e.g., Invitrogen iBlot 2 Transfer Stacks Cat #IB23001)
- ECL substrate

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) for Pan-Specific and α2,3-Specific Lectenz®
      ii. 5X SiaFind™ Binding Buffer 2 (Lectenz Bio Cat #BA0102) diluted to 1X + 0.1% Tween-20 (SBB2T) for α2,6-Specific Reagent
   b. Blocking Buffer: Wash Buffer + 5% BSA
   c. Biotinylated SiaFind™ Solution(s) prepared in Wash Buffer + 0.5% BSA:
      i. Pan-Specific Lectenz®: 20 μg/mL
      ii. α2,3-Specific Lectenz®: 5 μg/mL
      iii. α2,6-Specific Reagent: 0.2 μg/mL
   d. Secondary Solution: 1.0 μg/mL streptavidin-HRP conjugate (e.g., Vector Laboratories Cat #SA-5014-1) in Blocking Buffer
2. Prepare samples for Western blotting in 1X SDS-PAGE Sample Buffer. Analyte samples can be purified proteins, cell lysates, or any other protein containing solution.
3. Denature samples on a heating block for 5 min at 95°C.
4. Load samples along with a protein ladder on a precast acrylamide gel and run until the front of the indicator dye is at the bottom of the gel.
   a. Notes:
      i. The amount of sample loaded depends on the goal of your experiment and what components are in your samples. Usually, 0.1-10 μg of total protein per well is more than sufficient.
      ii. Load in duplicate or triplicate if more than one SiaFind™ probes (or other 1° probes) are to be evaluated.
5. Perform transfer according to transfer apparatus protocol.
6. After the transfer is complete, place the membrane in an incubation box containing Blocking Buffer.

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   a. Optional: Ponceau S stain and wash a section of the membrane to check for proper protein transfer prior to adding blocking reagent.
7. Incubate at room temperature for 1 h with gentle rocking.
8. Wash membrane with Wash Buffer for 3X 5 min with gentle rocking.
9. Perform primary incubation with SiaFind™ for 1 h probe at room temperature with gentle rocking.
10. Wash membrane with Wash Buffer for 3X 5 min with gentle rocking.
11. Perform secondary incubation with streptavidin-HRP conjugate for 1 h at room temperature with gentle rocking.
12. Wash membrane with Wash Buffer for 3X 5 min with gentle rocking.
13. Wash membrane with Binding Buffer for 3X 5 min with gentle rocking.
14. Transfer the membrane to a dry box, prepare just enough ECL substrate solution to cover the membrane. Expose/develop within 5 min.

Useful Tips:

- Make sure the glassware used is cleaned thoroughly. Leftover detergent can negatively impact blotting and result in high background.
- Make sure to avoid air bubbles during the transfer step. This can be done using a roller in most cases. Check your transfer using Ponceau S staining, if desired.
- Primary blotting reagents can yield different results depending on the membrane material on which the gel is transferred. SiaFind™ reagents are optimized for nitrocellulose. PVDF membrane tends to yield high background.
- ***Make sure to use SiaFind™ Binding Buffer (SBB1/SBB2) for SiaFind™ probes***, unless otherwise indicated on individual datasheet. Lectenz® reagents are salt sensitive and may not perform as well in PBS or TBS.
- Blocking and primary incubations can be performed overnight at 4°C if needed.
- High background can be reduced by:
    - Increasing BSA concentration in binding buffers from 0.5% up to 5% during primary and secondary incubations
    - Decreasing the amount of secondary detection reagent used
    - Increasing length and amounts of washing steps

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