Introduction:

This protocol is intended for general use with SiaFind™ reagents. However, it can also serve as a good, all-purpose blotting protocol if the buffers used are substituted for TBS, PBS, or other buffers depending on the detection reagents used.

Useful Tips:

- Make sure the glassware and membrane used is cleaned thoroughly
  - Leftover detergent can negatively impact blotting
- 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 staining, if desired.
- Primary blotting reagents can yield different results depending on the membrane material on which the gel is transferred
  - Our reagents are optimized for nitrocellulose, but do also work on PVDF
- ***Make sure to use SiaFind™ Binding Buffer (SBB1/SBB2) for SiaFind™ reagents.*** These reagents are salt sensitive and do 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 the BSA concentration in the primary and secondary incubation binding buffers from 0.5% up to 5%
  - Decreasing the amount of secondary detection reagent used
  - Increasing length and amounts of washing steps

Protocol:

1) Prepare samples for Western blotting in 1X SDS-PAGE Sample Buffer.
   a) Samples can be purified proteins, cell lysates, or any other protein containing solution
2) Denature samples on a heating block at 95°C for 5 min then spin in a microcentrifuge for ~1 min at max speed.
3) Load samples along with 5 µL of protein ladder on a precast acrylamide gel and run until dye front is at the bottom of the gel
   a) Notes:
      i) The amount of sample loaded depends on your experiment and what your sample consists of. Usually 0.1-10 µg of total protein per well is more than sufficient.
      ii) Load in duplicate or triplicate if more than one Lectenz® (or other 1° probes) are to be evaluated.
4) Perform transfer based on the system you are currently using

5) During transfer, prepare 1X Sia Lectenz Binding Buffer (SBB1/SBB2) and 1X Sia Lectenz Binding Buffer + 0.1% Tween-20 (SBB1T/SBB2T) by adding the enclosed 5X SLBB to 400mL MilliQ water.
6) After transfer is complete, transfer membrane to a membrane box
    a) Optional: Ponceau stain to check for proper transfer
7) Block membrane with SBB1T/SBB2T + 5% BSA at room temperature for 1 h, with gentle agitation
8) Wash membrane with SBB1T/SBB2T, 5 min × 3 times, with gentle agitation
9) Perform primary incubation with Lectenz probe for 1 hour at room temperature with gentle agitation
    a) Use 20 µg/mL for the pan-specific (SP0502B) in SBB1 + 0.5% BSA
    b) Use 5 µg/mL for the 2,3-specific (SP2302B) in SBB1 + 0.5% BSA
    c) Use 0.1 µg/mL for the 2,6-specific (SP2602B) in SBB2 + 0.5% BSA
10) Repeat Step **8**
11) Prepare Streptavidin-HRP secondary probe in SBB1T/SBB2T + 0.5% BSA at 1 ug/mL, supply to membrane, and rock for 1 h at room temperature
12) Repeat Step **8**
13) Wash with SBB1/SBB2 for 5 min × 3 times, with gentle agitation
14) Transfer membrane to a dry box, supply a Pico:ECL substrate mix just enough to cover the membrane. Expose/develop within 0-5 minutes.