Materials

- Humidity chamber
- Glass coverslips
- Coplin jar
- Pap pen
- 200 µL and 1000 µL tips
- 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
- Blocking Buffer (5% bovine serum albumin in Binding Buffer)
- 0.01% neutravidin or avidin in Binding Buffer, filtered
- 0.01% biotin in Binding Buffer, filtered
- Xylene
- 100% Ethanol
- 95% Ethanol
- 70% Ethanol
- 50% Ethanol
- Deionized (DI) water
- 0.3% H_2O_2 in either water or methanol
- Neuraminidase (e.g., Sialidase A, Agilent Cat #GK80040)
- Streptavidin-HRP (e.g., Vector Laboratories Cat #SA-5014-1, 1 mg/mL stock)
- DAB Peroxidase Substrate
- Hematoxylin, Gill's formulation
- Acid Rinse solution (2 mL glacial acetic acid + 98 mL of DI water, filtered)
- Bluing solution (1.5 mL NH_4OH (30% stock) + 98.5 mL of 70% ethanol, filtered)
- Aqueous Mounting Medium (e.g., Vector Laboratories Cat #H-5501-60)

Protocol

1. For paraffin sections, deparaffinize and rehydrate slides to water.
   a. To do this, prepare individual washes and perform as stated in the table below:

Solution	Duration
Xylene	3 min
Xylene	3 min
1:1 Xylene:100% Ethanol	3 min
100% Ethanol	3 min
100% Ethanol	3 min
95% Ethanol	3 min
70% Ethanol	3 min
50% Ethanol	3 min

   b. Rinse in DI water several times, until ethanol smell is gone. DO NOT LET SLIDES DRY OUT FROM THIS POINT ONWARD. To keep safe, place in humidity chamber.
2. Ensure humidity chamber is humidified.
3. Use the pap pen to draw a line on each slide to isolate tissue section.
4. (Optional) Perform the neuraminidase pre-treatment on half the slides: Apply a sufficient volume of neuraminidase to tissue sections. Incubate for 60 min at 37°C.
5. Rinse the tissue sections with Binding Buffer 3 times and place the slides in a Coplin jar with Binding Buffer for at least 5 min at room temperature.
6. Place slides in humidity chamber. Apply a sufficient volume of 0.3% H_2O_2 to completely cover all the sections. Incubate the slides for 15 min at room temperature.
7. Rinse the tissue sections with Binding Buffer 3 times and place the slides in a Coplin jar with Binding Buffer for at least 5 min.
8. Place slides in humidity chamber and apply sufficient Blocking Buffer to completely cover all the sections. Incubate for 30 min at room temperature to block the tissue sections.
9. Remove excess blocking solution from the tissue sections by tipping the slide and allowing the serum to drain off the edge of the slide and onto the bench paper.
10. Block endogenous biotin as follows:
    a. Incubate with neutravidin or avidin solution for 15 min.
    b. Briefly immerse in wash solution.
    c. Incubate with biotin solution for 15 min.
11. Rinse the tissue sections with Binding Buffer 3 times and place the slides in a Coplin jar with Binding Buffer for at least 5 min.
12. Place slides in humidity chamber and apply a sufficient volume of 80 µg/mL Pan-Specific, or 25 µg/mL α2,3-Specific, or 1 µg/mL α2,6-Specific reagent prepared in Blocking Buffer onto the tissue sections. Incubate for 1 h at room temperature or overnight at 4°C.
13. Rinse the tissue sections with Binding Buffer 3 times and place the slides in a Coplin jar with fresh Binding Buffer for at least 5 min at room temperature.
14. Place slides back in the humidity chamber and apply a sufficient volume of streptavidin-HRP (0.5-1 µg/mL) in Blocking Buffer to completely cover all the sections. Incubate for 30 min at room temperature.
15. Rinse the tissue sections with Binding Buffer 3 times and place slides in the Coplin jar with fresh Binding Buffer for at least 5 min.
16. Place slides back in the humidity chamber and apply a sufficient volume of DAB peroxidase substrate to completely cover all the sections. Incubate for 1-10 min at room temperature or until brown signal is observed. The signal produced by the SiaFind™ Pan-Specific and α2,3-Specific Lectenz® is weaker than that of the SiaFind™ α2,6-Specific Reagent (a lectin derivative), so longer incubations may be needed.
17. Counter-stain with hematoxylin to stain nuclei as follows:
    a. Rinse slides in DI water (or buffer).
    b. Cover sections for 1-5 min with hematoxylin.
    c. Rinse with DI water until sections are colorless.
    d. Dip slides 10 times in acid rinse solution followed by 10 times in water.
    e. Incubate slides in bluing solution for 1 minute followed by 10 dips in water.
18. Rinse the tissue sections 3 times with Binding Buffer and place slides in the Coplin jar with fresh Binding Buffer for at least 5 min.
19. Mount the tissue sections using aqueous mounting media and coverslip. Let set overnight.
20. Evaluate staining by light microscopy.