Lectin and Sialidase Treatment

Reagents

- Biotinylated SNA Lectin (Vector Labs, cat. #B1305)
- *Arthrobacter ureafaciens* Sialidase (AUS) (EY Labs, cat. #EC-32118-5) *get 5U size*
- Sodium Acetate ($NaCOOCH_3$)

Preparation of Reagents

1. 50 mM Sodium Acetate, pH 5.5
   a. Measure 100 mL Milli-Q water into a 250-mL beaker
   b. Add 0.681 g of sodium acetate and mix until dissolved
   c. Adjust pH to 5.5 using HCl or KOH
2. AUS Stock Solution (5mU/µL in 50 mM sodium acetate, pH 5.5)
3. AUS Aliquots
   a. Make 10 aliquots in labeled Eppendorf tubes (1 mL into 10 vials of 100 µL in each) and freeze at -20°C using 50 mM sodium acetate, pH 5.5
4. AUS Working Solution (250mU/mL)
   a. 50 µL of 5mU/µL AUS in 950 µL of sodium acetate

Procedure

1. Prepare two slides, both of which will receive biotinylated SNA lectin followed by labeled Streptavidin for detection of binding
   a. Slide 1 (SNA lectin only, no AUS): serves as positive control for tissue
   b. Slide 2 (SNA lectin followed by AUS): sialic acids are removed after treatment with AUS, and thus there will be no binding when using SNA, which will be the control for the AUS treatment of other slides
2. Get slides that are to be treated, overlay with 150 µL of working AUS solution, and overlay with coverslip and place on the top tray of pipette tip holder, the bottom of the box should contain wash buffer, so that when the box is covered, it forms a small humid chamber in which the sialidase treatment will occur at 37°C in the bacteriological oven, or hybridization oven.
3. Incubate the sections on the glass slides with AUS solution under a coverslip, in the humid chamber, for *at least **2.5 h*** at **37°C**.
4. Remove from the incubator, wash with washing buffer, and proceed with IHC.