Siallidase treatment of tissue sections

Sialidase (AUS) treatment protocol on tissue sections

Positive control for tissue: 2 slides needed which will both receive biotinylated SNA lectin followed by labeled Streptavidin for detection of binding

But treat one slide will be treated with AUS (as below) and the other slide will not be treated AUS

The Slide that receives only SNA lectin will be the positive control, and the
The Slide that receives AUS followed by the SNA lectin, will have the sialic acids removed after treating with AUS, and thus there will be no binding when using SNA, which will be the control for the AUS treatment of other slides.

Biotinylated SNA lectin : Vector Labs B1305
Enzyme: Sialidase Arthrobacter Ureafaciens (AUS) from EY Labs Cat # EC-32118-5. Purchase a 5 Unit vial
Make a Stock solution of AUS: Make a stock of 5mU/uL in 50 mM sodium acetate pH 5.5 and aliquot at minus 20^{o}C

Solutions needed:

1. Make 50mM Sodium acetate
    a. Place 100 ml of MQ water in a 250 ml container
    b. Add 0.681 g of $NaCOOCH_3$ and mix until dissolved
    c. Use the pH meter to pH to 5.5 using HCl or KOH
2. Make aliquots of AUS
    a. Purchase AUS from EY labs # EC-32118-5 at a 5 Unit size
    b. Make aliquots in eppendorf tubes of 5mU/uL of the AUS (1 milliLiter into labeled 10 vials of 100 microLiter in each) and freeze at minus 20^{o}C using 50milliM sodium acetate pH 5.5
3. Prepare working solution of AUS in sodium acetate pH 5.5
    a. Dilute one 5milliU/microLiter aliquot with 50 mM sodium acetate to get 250milliU/milliL
    eg: 50 microL of 5milliU/microL in 950 microL of sodium acetate.
4. Get slides that are to be treated, overlay with 150 microL 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^{o}C in the bacteriological oven, or hybridization oven.
5. Incubate the sections on the glass slides with AUS solution under a coverslip, in the humid chamber, **at 37 ^{o}C, for at least 2.5 hours**
6. Remove from incubator, wash in washing buffer and proceed with IHC.