Immunohistochemistry on cryosections using TSA Plus Fluorescence kits

(adapted from *TSA Plus Fluorescence Kit Manual*)

1. Slide Preparation

a) Air dry slides in oven for 30 min -1 hour.

b) Wash the slides in 1XPBS for 5 min.

c) Quench endogenous peroxidase activity by incubating the slides in 3% H_2O_2 in PBS for 10 min.

d) Wash the slides in 1XPBS for 5 min.

2. Antigen retrieval (Heat-based antigen retrieval)

a. Preheat the steamer

b. Preheat the antigen unmasking working solution in a microwave for 10 mins or by heating the solution over a Bunsen burner until boiling

Note: prepare 450ml antigen unmasking working solution (4.2ml antigen unmasking stock solution + 450 ml dH_20)

c. Transfer the boiled antigen unmasking solution to the steamer and steam the slides in the antigen unmasking solution for ~15-20mins

d. After steaming is complete, transfer the slides to FRESH 1XPBS solution

3. Blocking Step

Cover tissue sections with TNB blocking buffer and incubate slides in a humidified chamber for 60 minutes at room temperature.

TNB Blocking Buffer

0.1 M TRIS-HCl, pH 7.5

0.15 M NaCl

0.5% Blocking Reagent

4. **Primary Antibody Incubation**
    a) Drain off the blocking buffer
    b) Dilute the primary antibody in TNB blocking buffer (concentration depends on the antibodies used)
    c) Add primary antibody to the sides. Use enough volume to completely cover the tissue section (generally 100-300 µL per slide).
    d) Incubate the primary antibody for 60 minutes at room temperature.

5. **Wash**

    Wash the slides 3X for 5 minutes each in TNT Buffer at room temperature.

    TNT Wash Buffer
    1M TRIS-HCl, pH 7.5
    0.15 M NaCl
    0.05% Tween®20

6. **Introduction of HRP**
    a) Drain off the blocking buffer
    b) Dilute the HRP labeled secondary antibody in TNB blocking buffer at 1:200-1:500
    c) Add primary antibody to the sides. Use enough volume to completely cover the tissue section (generally 100-300 µL per slide).
    d) Incubate the slices in HRP labeled secondary antibody for 1-2 hours at room temperature.

7. **Wash**

    Wash the slides 3X for 5 minutes each in TNT wash buffer at room temperature

8. **Amplification**
    a) Pipette 100-300 µL of TSA Plus Working Solution onto each slide.
    b) Incubate the slides at room temperature for 3 to 10 minutes.

9. **Wash**

    Wash the slides 3X for 5 minutes each in TNT wash buffer at room temperature.

10. **Visualization of Deposited Fluorophores**

    For fluorescent detection, counterstain with DAPI and mount for fluorescence microscopy.