Immunofluorescence staining using TSA Fluorescein Signal Amplification from Perkin Elmer (NEL701A001KT)

Clearing and rehydration of tissue sections:

- Incubate slides at 55°C for 30 minutes to allow paraffin sections to slightly soften and flatten on slide.
- Perform clearing and rehydration of tissue sections:
  1. Xylenes: 3 minutes
  2. Xylenes: 3 minutes
  3. Xylenes: 3 minutes
  4. 100% Ethanol: 3 minutes
  5. 100% Ethanol: 3 minutes
  6. 100% Ethanol: 3 minutes
  7. 95% Ethanol: 1 minute
  8. 80% Ethanol: 1 minute
  9. 70% Ethanol: 1 minute
  10. Milli-Q water: 5 minutes

Antigen Retrieval:

- Tris-EDTA antigen retrieval (10mM Tris Base, 1mM EDTA, 0.05% Tween20 pH9.0) or 10mM Sodium Citrate pH 6.0 (10mM Sodium Citrate, 0.05% Tween20, pH 6.0) for 20 minutes. Incubate slides for 5 minutes on “HIGH” power and then reduce to 50% power for the remaining 15 minutes. This is microwave dependent, but let the buffer get to a low simmer before lowering the power to 50%.
  - Preparation of 10X Tris EDTA pH 9.0 buffer:
    - Tris Base..............12.1.g
    - EDTA................3.7g
    - Milli-Q water........q.s. to 1000mL
    Mix to dissolve. pH is usually at 9.0 and then add 5 mL of Tween 20 and mix well. Store at 4°C for up to 6 months. Dilute 1:10 before use.
  - Preparation of 10X Sodium Citrate pH 6.0 buffer:
    - Tri-sodium citrate (dihydrate).........29.4g
    - Milli-Q water.............................1000ml
    Mix to dissolve. Adjust pH to 6.0 with 10N NaOH and then add 5ml of Tween 20 and mix well. Store at 4°C for up to 6 months. Dilute 1:10 before use.
- Cool slides for 30 minutes.
- Wash slides twice with 1X PBS for 5 minutes each.
- Block endogenous peroxidase activity (for TSA only) by incubating in 3% H₂O₂ for 10 minutes at room temperature.
- Wash slides in 1X PBS for 5 minutes.

Immunostaining:

- Using a PAP pen, circle each tissue section and apply 75 µl of blocking buffer to each section. Do not let sections dry out. **Block for 1 hr. at room temperature.**
o For most species (except mouse): 5% BSA in 1X PBS or 5% goat serum in 1X PBS is appropriate as the block and antibody dilution buffers.

  o For mouse monoclonal primary antibodies, use MOM block.
    ▪ MOM block preparation: 2 drops of MOM block into 2.5mL of 1X PBS.

  o NOTE: If performing co-staining, dilute MOM block in 1X PBS with 5% BSA or 5% goat serum (same for MOM diluent etc.).

➢ Wash with 1X PBS-T (1X PBS with 0.05% Tween-20) for 2 minutes.

➢ Incubate sections with primary antibody overnight at 4°C.

  o Dilute primary antibody in primary antibody dilution buffer or MOM diluent. ** Generally, primary antibodies can be diluted at least 5-fold when performing TSA-amplification steps. Antibody titration should be performed.
    ▪ MOM Diluent is prepared by adding 600 µl of MOM diluent to 7.5 mL of 1X PBS.
    ▪ NOTE: If performing co-staining, dilute MOM diluent in 1X PBS with 5% BSA.

➢ The next day, aspirate primary antibody and wash sections 3X with 1X PBS-T (0.05% Tween-20) for 5 minutes each.

➢ Incubate sections with secondary antibody for 1 hr. at room temperature.

  o For antigen of interest to be TSA-amplified, dilute biotinylated secondary antibody 1:1000 (Vector labs) in appropriate volume of dilution buffer.

  o If performing co-staining, add conjugated fluorescent secondary (Alexa conjugate) to this step at a 1:1000 dilution.

➢ Wash sections 3X in 1X PBS-T for 5 minutes each.

➢ Incubate sections with SA-HRP (from TSA kit) diluted 1:100 in appropriate dilution buffer (5% BSA in 1X PBS etc.) for 30 minutes.

➢ Wash sections 3X in 1X PBS-T for 5 minutes each.

➢ Prepare TSA Plus Working Solution: dilute TSA plus stock solution 1:50 in 1X amplification diluent to make TSA plus working solution. Use about 75 µl TSA plus working solution per section.

  o ***Incubate slides at room temperature for a minimum of 3 minutes. Optimal incubation times will vary depending on antigen of interest and primary antibody dilution.

➢ Wash slides 3X with 1X PBS-T for 5 minutes each.

➢ Counter stain with DAPI for 3 minutes.

➢ Mount in Aqua Poly Mount.

➢ Allow slides to dry overnight.