

Date: _____

Time point: _____

of ears: _____

TSA Math1 immuno protocol:

- Fix 1 hour in 4% paraformaldehyde in PBS.
- Rinse 3x in PBS, 5 minutes each rinse.
- Permeabilize tissue in 0.17% Triton X-100 in PBS for 1 hour at 4°C.
200 μ L 1% Triton X-100 in 1 mL PBS
- Block endogenous peroxidases with 0.4% H₂O₂ in PBS for 1 hour at 4°C.
15 μ L 30% H₂O₂ in 1 mL PBS
- Rinse 3x in PBS, 5 minutes each rinse.
- Block non-specific antibody binding in 1% NGS in PBS for 30 minutes at 4°C.
100 μ L 10% NGS in 900 μ L PBS
- Primary antibody (1:200) in 1% block: _____ overnight at 4°C.

- Rinse 3x in PBS, 5 minutes each rinse.
- HRP conjugated secondary antibody (1:100) in 1% block for 2 hrs at 4°C.
- Rinse 3x in PBS, 5 minutes each rinse.
- Prepare TSA reagent:
1 μ L 30% H₂O₂ with 200 μ L amplification buffer
1 μ L of the above in 100 μ L of amplification buffer (now 0.0015% in buffer)
add 1 μ L TSA per 100 μ L of 0.0015% H₂O₂ dilution
- Add TSA reagent to tissue for 10 minutes in the dark at room temperature.
- Rinse 3x in PBS, 5 minutes each rinse.

Optional:

- Counterstain: _____ for _____.
- Rinse 3x in PBS, 5 minutes each rinse.

- Nuclear label: _____ for _____.
- Rinse 3x in PBS, 5 minutes each rinse.
- Mount tissue