

Date: \_\_\_\_\_

Time point: \_\_\_\_\_

# of ears: \_\_\_\_\_

**Immuno protocol:**

**Day1:**

- Fix 1 hour in 4% paraformaldehyde in PBS.
- Rinse 3x in PBS, 5 minutes each rinse.
- Permeabilize tissue in 1% Triton X-100 in PBS for 10 minutes.
- Rinse 3x in PBS, 5 minutes each rinse.
- Block in 10% NGS in PBS for 10 minutes.
- Rinse 1x in PBS for 5 minutes.
  
- Primary Antibody in PBS: \_\_\_\_\_ for \_\_\_\_\_.
- Rinse 3x in PBS, 5 minutes each rinse.
- Secondary antibody in PBS: \_\_\_\_\_ for \_\_\_\_\_.
- Rinse 3x in PBS, 5 minutes each rinse.

**Day 2:**

- Primary Antibody in PBS: \_\_\_\_\_ for \_\_\_\_\_.
- Rinse 3x in PBS, 5 minutes each rinse.
- Secondary antibody in PBS: \_\_\_\_\_ for \_\_\_\_\_.
- 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