

Date: _____

Time point: _____

of ears: _____

For Sox2 labeling with Ab#1569 (made in goat)

Immuno protocol:

- 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% **NDS (Normal Donkey Serum)** in PBS for 10 minutes.
- Rinse 3x in PBS for 5 minutes.

- Primary Antibody in PBS: Santa Cruz Sox-2 Y-17 (goat) 1:500 for ON @ 4°C .
- Rinse 3x in PBS, 5 minutes each rinse.
- Secondary antibody in PBS: Alexafluor 568 Donkey anti-goat 1:5000 for 2h @ RT.
- Rinse 3x in PBS, 5 minutes each rinse.

Optional:

- Counterstain: Phalloidin 488 in PBS 1:200 _____ for 1h @ RT .
- Rinse 3x in PBS, 5 minutes each rinse.

- Nuclear label: DAPI in antifade
- Mount tissue