

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

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

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

### EdU/Antibody NDS immuno protocol:

#### Antibody Labeling:

- 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, 10 minutes each rinse.
- Block in 10% NGS in PBS for 10 minutes.
- Rinse 3x in PBS, 5 minutes each rinse.
  
- Primary antibody in PBS: \_\_\_\_\_ for \_\_\_\_\_.
- Rinse 3x in PBS, 5 minutes each rinse.
- Secondary antibody in PBS: \_\_\_\_\_ for \_\_\_\_\_.

#### EdU Labeling:

- The buffer additive is stored as a 10X solution; once it is thawed, dilute enough with dH<sub>2</sub>O for your purposes and put on ice.
- At the third rinse, start to make up the reaction cocktail, adding the azide last; *use the reaction cocktail within 15 minutes*
- Use either the Alexa Fluor 488 or 568 azide.

TOTAL VOLUME	200 $\mu$ L	400 $\mu$ L	600 $\mu$ L	800 $\mu$ L	1000 $\mu$ L
Reaction Buffer	175 $\mu$ L	350 $\mu$ L	525 $\mu$ L	700 $\mu$ L	875 $\mu$ L
Buffer Additive	20 $\mu$ L	40 $\mu$ L	60 $\mu$ L	80 $\mu$ L	100 $\mu$ L
CuSO <sub>4</sub>	4 $\mu$ L	8 $\mu$ L	12 $\mu$ L	16 $\mu$ L	20 $\mu$ L
Azide	1 $\mu$ L	2 $\mu$ L	3 $\mu$ L	4 $\mu$ L	5 $\mu$ L

- Incubate in EdU cocktail for 30 minutes.
- Rinse 3x in PBS, **15 minutes** each rinse.
- Mount tissue