Cochlear Whole Mount Stain Protocol

Acronyms or shorthands:

EDTA ethylene diamine tetraacetic acid
Fab AffiniPure Fab Fragment Donkey Anti-Mouse IgG
PBS phosphate buffered salts
PFA paraformaldehyde
RT room temperature

Recipes:

4% PFA in 1xPBS: 10mL 16% PFA + 4mL 10xPBS, up to 40 mL total volume with ddH_2O
120 mM EDTA in 1xPBS: 240mL 0.5M EDTA + 100mL 10xPBS, up to 1L total volume with ddH_2O
1xPBS with 0.01% sodium azide: 100mL 10xPBS + 5g sodium azide, up to 1L total volume with ddH_2O
30% sucrose in 1xPBS: 300g sucrose + 100mL 10xPBS, up to 1L total volume with ddH_2O
1% PBST: 1mL Triton-x + 10mL 10xPBS, up to 100mL total volume with ddH_2O
0.3% PBST: 300µL Triton-x + 10mL 10xPBS, up to 100mL total volume with ddH_2O
0.02% PBST: 20µL Triton-x + 10mL 10xPBS, up to 100mL total volume with ddH_2O

Preparing adult cochlear tissues for staining:

1. Perfuse adult mouse with 4% PFA in 1xPBS.
2. Quickly extract inner ear from temporal bone and prick bony wall close to the basal turn.
3. Fix ear at **RT** for **1 hr**.
4. Wash with 1xPBS.
5. Decalcify ear at **4°C** for **48h** in 120 mM EDTA in 1xPBS.
6. Wash with 1xPBS.
7. Dissect three cochlear turns in 1xPBS—removing as much spiral ligament as possible.
8. Store in 1xPBS in short term or 1xPBS with 0.01% sodium azide in the long term.

Best practices for stain:

- Use 2-mL tubes for the stain.
- Couple a 1-mL pipette tip with 200-µL pipette tip to aspirate solutions gently around turns.
- Crush dry ice right before use.

Day 1 of stain

Protease III Permeabilization (Optional – targets OSL)

1. Add ~500 µL of Protease III. Rotate at **37°C** for **30 min**.
2. Wash with 1xPBS at **RT** for **5 min**, thrice.

Sucrose Permeabilization (Optional – targets synapses)

1. Add 1 mL of 30% sucrose in 1xPBS. Rotate at **RT** for **20 min**.
2. Freeze in dry ice for **10 min**.
3. Thaw quickly in water at **RT**.
4. Wash with 1xPBS at **RT** for **5 min**, thrice.

Blocking

1. Block with 500 µL of 10% normal serum in 1% PBST. Rotate at **RT** for **1 hr**.
2. Wash with 0.02% PBST at **RT** for **5 min**.
3. If planning to use a mouse antibody, block with 500 µL of 1:25 Fab in 0.3% PBST. Rotate at **RT** for **1 hr**. Wash with 0.02% PBST at **RT** for **5 min**, thrice.

Primary Antibody Incubation

1. Prepare primary antibody mix with 1% NGS, 1%NDS in 0.3% PBST.
2. Spin primary mix at **14,000 RPM** for **15 min** to remove particulate matter.

3. Add 500 µL of primary mix. Rotate at **37°C**, **overnight**.

Day 2 of stain

4. Wash with 1% PBST for **15 min**, thrice.

Secondary Antibody Incubation

1. Prepare secondary antibody mix with 1% serum, 0.3% PBST.
2. Spin secondary mix at **14,000 RPM** for **15 min** to remove particulate matter.
3. Add 500 µL of secondary mix. Incubate on rotator in **37°C overnight**.

Day 3 of stain

4. Wash with 1% PBST for **15 min**, thrice.