Published in:

McLean et al. 2009. *J Assoc Res Otolaryngol*. Distribution of the Na,K-ATPase alpha subunit in the rat spiral ganglion and organ of corti. 10(1):37-49. [auditory epithelium]

Schuth et al. 2014. *J Assoc Res Otolaryngol*. Distribution of Na,K-ATPase α subunits in rat vestibular sensory epithelia. 15(5):739-54. [vestibular epithelia]

Instructions:

1. Remove inner ear from temporal bone in ice cold PBS
2. Clear round and oval windows of excess tissue and chip an opening in bony covering of apical cochlear turn in PBS

**An excellent video tutorial is available at http://www.jove.com/video/2442/postsynaptic-recordings-at-afferent-dendrites-contacting-cochlear (start with "Dissecting the cochlear tissue sample").*

3. For isolation of the organ of Corti: transfer inner ear to petri dish containing ice cold 4% PFA/PBS and use a syringe with 25 gauge needle to push approximately 3 mL of ice cold 4% PFA/PBS through cochlear windows
   **Or simply transfer inner ear with apical turn exposed to approximately 5 mL of ice cold 4% PFA/PBS.*

   For isolation of the vestibular sensory epithelium (utricle (the utricular macula) and the horizontal and anterior canals (the semicircular canal cristae), remove the bony covering encasing the epithleia (where the vestibular nerve exits) but allow the epithelia to remain attached (otherwise they will curl up when placed into PFA)

4. Place inner ear in approximately 5 mL of ice cold 4% PFA/PBS for 30 min to 3 h
5. Remove inner ear and place in ice cold PBS
6. Isolate organ of Corti by removing bone, stria, and tectorial membrane and then lifting free from modiolus
7. Place organ(s) of Corti into ice cold PBS in a 24-well culture dish
8. Block for 1 to 2 h (or O/N) in blocking buffer

   **Use micropipets to exchange solutions; 300 µL is the minimal volume we typically use in 24-well culture dishes*
   **We routinely do all incubations and rinses on a rocking platform at room temperature (even O/N incubations).*

9. Incubate 4 h (or O/N) in primary antibody diluted in blocking buffer
10. Wash 3 times 10 min in PBT

11. Incubate 1 to 2 hours in secondary antibody diluted in blocking buffer
12. Wash 3 times 10 min in PBT
13. Rinse 1 time in PBS
14. Mount in Vectashield mounting medium

Recipes:

- 4% PFA/PBS: Dilute 16% or 32% PFA in appropriate amount of 10X PBS and water (no need to pH)

- Blocking buffer: 5 % Normal goat serum (or serum from host of secondary antibody) with 0.6 % TX-100 and 0.5 % saponin in PBS OR 1 % BSA with 0.6 % TX-100 and 0.5 % saponin in PBS
  **More recently we have increased the concentration of TX-100 to 4%.*

- PBT: PBS with 0.6 % TX-100

Reagent vendors:

- PFA (16% or 32%): Electron Microscopy Sciences (http://www.emsdiasum.com/microscopy/) 15710 or 15714 (10 x 10 mL ampoules)
- Normal goat serum: Vector Labs (http://www.vectorlabs.com/) S-1000
- Vectashield: Vector Labs (http://www.vectorlabs.com/) H-1000 (also available with DAPI)
- Secondaries: Invitrogen/Molecular Probes (http://igene.invitrogen.com/antibody/browse/secondary-antibodies/1/target0/conjugate/alexa-fluor-dyes)

Example combinations of secondaries:

Alexa Fluor® 488 goat anti-mouse IgG (H+L) *2 mg/mL* (A-11001) *and*
Alexa Fluor® 594 goat anti-rabbit IgG (H+L) *2 mg/mL* (A-11012)

Alexa Fluor® 488 goat anti-mouse IgG_1 (γ1) *2 mg/mL* (A-21121) *and*
Alexa Fluor® 568 goat anti-rabbit IgG (H+L) *2 mg/mL* (A-11011) *and*
Alexa Fluor® 647 goat anti-mouse IgG_{2a} (γ2a) *2 mg/mL*(A-21241) *or*
Alexa Fluor® 647 goat anti-mouse IgG_{2b} (γ2b) *2 mg/mL*(A-21242)