Mouse cochlear culture preparation

Supplies:

DMEM/F12 medium (Sigma D8062)
Foetal calf serum (any supplier)
Cell-Tak (BD Bioscience, BD 354240)
HBSS (GIBCO 14025-050)
Hepes 1M (Sigma, H0887)
0.1 M sodium bicarbonate pH 8.0 (sterile filtered)
Mice (postnatal days 0-3)
Mat-Tek dishes (MatTek Corporation, Cat # P50G-0-14-F)
Ampicillin (Sigma A-9518; 10 mg/ml in H2O, sterile filtered)
90 mm and 35 mm diameter sterile plastic petri dishes (any supplier)

Equipment:

Horizontal lamina air hood
Dissecting microscope with fibre optic light source
Dissecting instruments
CO2 incubator

Procedure:

1. Prepare medium: 93 ml DMEM/F12, 7 ml FCS, 100 ul ampicillin (10mg/ml).
2. Prepare Hepes buffered Hanks' Balanced salt solution (HBHBSS): Add 5 ml 1 M Hepes to 500 ml HBSS.
3. Prepare Cell Tak coated Mat Tek dishes: To 20 ul Cell Tak add 300 ul 0.1 M sodium bicarbonate pH 8.0; immediately add 60 ul to each well and spread. Replace lid; do not let the Cell Tak dry out. Wash 2 with HBHBSS before adding tissue (see below).
4. Kill mouse pups via approved method.
5. Surface sterilize pups by immersion in 80% ethanol for 6 min (3 changes, 2 min each).
6. Cut of heads and drop into 90 mm diameter dish containing HBHBSS (if using mutants remember to snip the tail and freeze for subsequent genotyping).
7. Bissect heads in two along the mid-saggital plane.
8. Transfer half heads to 90 mm diameter dish with HBHBSS.
9. Remove brain, pop out the cochleae from half heads, separating them from the vestibular bit of the labyrinth and place in 35 mm diameter dish with HBHBSS.
10. Remove cartilagenous capsule and transfer (with forceps) the still coiled cochlea complete (if possible) with stria vascularis to a 35 mm diameter dish with HBHBSS.
11. Remove (unwind) the stria, and separate the cochlear coil (GER/LER complex) from the mesenchymal modiolar tissue without unwinding or stretching the epithelium.
12. Cut cochlear coils into basal and apical 'halves' (cochlear is only 1.5 to 1.75 coils at this stage) with a pair of sharp needles.
13. Transfer basal and apical coils (with a serum prewetted, bent glass Pasteur pipette, or with a curette [a small spoon]) to a dish of clean HBHBSS.
14. Transfer coils to the medium filled wells (200 ul per well) of the Mat-Tek dishes with a curette, making sure the coils are sunny-side (hair-cell side) up before sliding them out of the spoon onto the substrate.

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