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Mouse Olfactory Horizontal Basal Cell Culture

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We use this protocol and it's working

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Abstract

This protocol describes isolation and culture of basal cells from the olfactory epithelium of mice. The process involves serial enzymatic treatment of dissected olfactory epithelia and subsequent plating in commercial airway medium with dual SMAD inhibitors. The resulting cultures express the horizontal basal cell markers CK5 and CK14.



Materials

Dissection medium:

- Pneumacult-Ex medium
- 1x B27 supplement without vitamin A
- 1x N2 supplement
- 1% penicillin/streptomycin

HBC medium:

- Pneumacult-Ex medium
- 1x Glutamax
- 1x B27 supplement without vitamin A
- 1x N2 supplement
- 1% pen/strep
- 10ng/mL TGF alpha
- 1 uM A-83-01



- 1uM DMH-1



- 1 Dissect olfactory mucosa from nasal septum  On ice .
Induce anesthesia via intraperitoneal injection of 0.15 mL euthanyl, followed by decapitation. Peel off skin and excise the eyes and the jaw. Disinfect the skull with 70% ethanol and carefully bisect along the sagittal plane, maintaining a 1 mm distance from the interfrontal suture. Transfer the larger segment of the skull under a dissection microscope, dissect a 1 mm distal section from the nasal bone. Carefully remove the nasal bones and collect the olfactory epithelium surrounding the septal cartilage into Hank's Balanced Salt Solution.
- 2 Mince the tissue in  2.7 mL dissection medium, add  300 μ L 10x Collagenase/Hyaluronidase and transfer to a 15 mL falcon.
- 3 Briefly vortex falcon and rotate at  37 °C for 1h.
- 4 Spin down at  80 x g for 30s and discard supernatant.
- 5 Triturate pellet in  5 mL TrypLE for 2 min and add  10 mL cold HBSS.
- 6 Spin down at  350 x g for 5 min and resuspend cells in 1 mL warmed dispase and 50 μ L DNase 1. Triturate for 2 min.
- 7 Add  10 mL cold HBSS and filter through 40 μ m cell strainer.
- 8 Spin down at  350 x g for 5 min, discard supernatant, resuspend in HBC medium with  10 micromolar (μ M) ROCK inhibitor Y27632.
- 9 Plate on PDL and laminin coated wells, one mouse septum on 1.9 cm² (1 well in a 24-well plate)
- 10 10. Refresh medium the next day without ROCK inhibitor.



- 11 11. Feed every 2 days. Passage with accutase and do not use any other cell dissociation products such as TrypLE or trypsin. Cultures are homogenously CK5+ after 3 passages.