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Olfactory Organoids

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ASAP Collaborative Rese…

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We use this protocol and it's working

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Abstract

This protocol enables obtaining 3D matrix-embedded olfactory organoids from KRT5+ olfactory horizontal basal cells. Resulting samples consist of hydrogel matrix domes with a volume of 40 µL containing hollow spheres of olfactory epithelium. The amounts were given per sample and should be calculated according to how many samples will be made.

Materials

Organoid initiation medium:

- 1:1 mixture of Advanced DMEM and Neurabasal A media
- 1x B27 supplement
- 1x N2 supplement
- 1% Glutamax
- 1% penicillin/streptomycin
- 3 µM CHIR99021
- 500 ng/mL R-Spondin 1
- 1.5 µM DMH-1
- 1.5 µM A83-01

Maintenance medium:

- 1:1 mixture of DMEM/F12 and Neurabasal A media
- 1x B27 supplement
- 1x N2 supplement
- 1% Glutamax
- 1% penicillin/streptomycin
- 3 µM CHIR99021
- 1 µM DMH-1
- 1 µM A83-01

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 Thaw reduced growth factor matrix precursor aliquots at 4 °C overnight. 20 µL matrix precursor is required per sample. Geltrex and Matrigel were tested and shown to work equally well with this protocol. Keep these products at 4 °C or on ice throughout the protocol.

2 Place 24-well plates into tissue culture incubator at 37 °C to equilibrate the temperature of the plastic. This step is important to prevent sinking of the cells to surface inside the matrix precursor.

3 Lift olfactory basal cells from the culture surface by rinsing with dPBS and adding cold accutase. Incubate at 37 °C for up to 15 minutes.

4 Add HBC medium equal to the volume of accutase and transfer the cells into a falcon tube. Centrifuge at 350 x g for 4 minutes and discard the supernatant. Resuspend cells in 2 mL HBC medium and perform cell count.

5 Transfer 4x10^4 cells per sample into a new falcon tube and centrifuge at 350 rpm . Discard supernatant. Add 15 µL organoid initiation medium per sample and resuspend the cells. Perform the following steps as quickly as possible.

6 Add 30 µL matrix precursor per sample to the cell suspension and briefly triturate to homogenize. Retrieve 24-well plates from the incubator and pipette 40 µL of the cell suspension into the center of each well, forming a liquid dome. Immediately return the well plates to the incubator. Aim to finish pipetting and return the plates to the incubator within 3-4 minutes after adding the matrix precursor to the cells.

7 Allow the matrix solutions to set in the incubator for 30 minutes. Confirm that the cells did not settle to the bottom using an inverted microscope. Gently fill the wells with 500 µL organoid initiation medium by pointing the pipette tip to the well wall.

8 Return the well-plates to the incubator. This day is denoted as 0 days-in-vitro (0 DIV).

9 At the end of 4 DIV, carefully aspirate the organoid initiation medium and add 500 µL maintenance medium. At this stage, cell clumps and hollowing spheres should be observed under the microscope.

10 Over the following days, spheres should start to hollow out. At the end of Day 7, supplement the maintenance medium with [M] 3 micromolar (µM) retinoic acid. The sphere walls will then start to thicken and differentiate into pseudostratified architecture of the olfactory epithelium. The samples are ready for further testing by 20 DIV.