Airway Epithelium Isolation

This document describes the recommended protocol for isolation of primary human airway epithelial cells from a section of trachea using CnT-Prime Airway (CnT-PR-A) medium.

This protocol suggest the seeding of small cell clumps/organoids directly after dispase separation, thereby avoiding the additional digestion step to produce single cells.

For the recommended thawing, passaging, and freezing protocols, please see the General Cultivation Protocol in the resources section of www.cellntec.com.

Preparation

Before starting to work with the cells, ensure that any medium, buffers or enzymes required have been prepared at the recommended concentrations.

Always use sterile instruments, aseptic technique, and work in a laminar flow to maintain sterility.

Isolation Protocol

1. Wash trachea in PBS, and cut into small pieces, ~1.0 x 1.0 cm
2. Incubate with 1x dispase (CnT-DNP-10) in CnT-PR-A at 4°C for 15-18 h
3. Transfer tissue pieces into separate Petri-dishes, in a small volume of CnT-PR-A (~3 mL)
4. Separate epithelium from stroma by gently rubbing tissue pieces with curved forceps – dislodged epithelial tissue will float away, and can be transferred to a conical tube
5. Pipette up and down in the conical tube to break up epithelial pieces into small cell clumps
6. Seed cell clumps into a surface area approximately 10 x larger than the surface area of the starting piece of trachea
7. Culture cells in CnT-PR-A supplemented with IsoBoost (CnT-ISO-50) for at least the first 3 days post seeding, then switch to standard CnT-PR-A medium. Change medium every 2-3 days.
8. Passage cells before they reach confluency, or when the older cells in the center of the colonies begin to change morphology (approximately 2'000 cells per colony). Accutase is recommended for passaging, because it is gentle, and does not need a separate reagent to stop the reaction
9. After passaging, seed viable cells at a density of 4 x 10^3 cells / cm^2 in culture vessels.
10. Culture the cells at 37°C and 5% CO_2.

For additional culture protocols (passaging, freezing, thawing) please visit the resources section of www.cellntec.com.

In case of questions, please email out scientists directly: scientist@cellntec.com