Cornea Epithelium Isolation

This document describes the recommended protocol for isolation of primary human corneal epithelial cells using CnT-Prime (CnT-PR) medium.

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 the medium, buffers and enzymes required are prepared at the desired concentration.

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

Isolation Protocol

1. Carefully dissect the disc of corneal tissue from the eye. Ensure that the limbal region is included.
2. Store the tissue at 4°C until early evening before starting isolation.
3. Place the tissue pieces in a 15 mL centrifuge tube containing 10 mL of 1x dispase solution (prepared from CnT-DNP-10), and 2 x antibiotics / antimycotics (CnT-GAB10).
4. Incubate the tisse over night (~ 15 h) at 4°C in a horizontal orientation.
5. Next day transfer the tissue together with the dispase solution into a Petri dish.
6. Transfer each tissue piece to a new Petri dish containing CnT-PR medium to wash away excess dispase.
7. While holding the tissue submerged in CnT-PR medium, gently separate the stroma (pink, opaque, gooey) from the epidermis (whitish, semi-transparent) with two pairs of curved forceps. If the separation does not occur easily, put the tissue back into the Petri dish with dispase solution for another 60 min at room temperature.
8. Transfer the epithelial sheets into 2 mL of Accutase (CnT-Accutase-100), to separate the epithelial cells
9. Incubate for 10-15 min at 37°C (cover the Petri dish with the lid to prevent evaporation).
10. Gently pipette up and down to break up the epithelial layer. If digestion is not complete, allow another 5 minutes of Accutase treatment.
11. Transfer the single cell solution to a 15 mL centrifugation tube, and add 5 mL of CnT-PR medium to dilute the Accutase.
12. Centrifuge the for 5 min, 180 x g at room temperature.
13. Aspirate the supernatant and resuspend the cell pellet in 2 mL CnT-PR medium.
14. Count the cells, and seed viable cells at an initial density of 4 x 10^4 cells / cm^2.
15. Culture cells in CnT-PR supplemented with IsoBoost (CnT-ISO-50) for at least the first 3 days post seeding, then switch to standard CnT-PR medium. Culture the cells at 37°C and 5% CO_2.

For subsequent 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