Bladder Epithelium Isolation

This document describes the recommended protocol for isolation of primary human urothelial cells from a bladder biopsy using CnT-Prime (CnT-PR) 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. Place the bladder tissue sample directly into CnT-PR medium after removal. The tissue may be stored at 4°C until ready to proceed with step 2.

2. Place the tissue into a Petri-dish. Cut it in 1 x 1 cm size pieces using a sterile scalpel.

3. Place the tissue pieces in a 15 mL centrifuge tube containing 10-12 mL dispase solution 1x (made from CnT-DNP-10 with CnT-PR medium and 2x antibiotics / antimycotics (cat # CnT-GAB-10).

4. Incubate overnight (~15 h) at 4°C. Ensure good tissue – dispase contact by placing the tubes in a horizontal orientation. Ensure that the pieces are well distributed in the tube.

5. Pour the tissue together with the dispase solution into a Petri dish.

6. Transfer each tissue piece to a new Petri dish containing CnT-PR to wash away excess Dispase.

7. It may be difficult to visually determine the epithelial part of the tissue samples. To remove the epithelium, scrape all surfaces of the tissue piece with curved forceps. Separated epithelial cells will appear as white conglomerates, the remaining stroma will appear reddish.

8. Transfer the epithelial cell conglomerates into a 15 ml tube using a 1 ml Gilson pipette. Avoid transferring more than 1 mL of medium with the cells. Large conglomerates/tissue pieces may be broken up by pipetting up and down several times.

9. We do not recommend further enzymatic disaggregation of these cell conglomerates, because subsequent cell attachment and outgrowth is easily impaired. Seed the conglomerates directly into culture flasks with an area approximately 5-10 times larger than the original tissue sample.

10. Culture the cells in CnT-PR medium supplemented with IsoBoost (CnT-ISO-50) for at least 3 days, then switch to regular CnT-PR medium. Change medium 2 days later and then every 3rd day. (If the culture is confluent the next day due to seeding at a very high density, change medium in the morning and passage in the afternoon).

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