Melanocyte Isolation

This document describes the recommended protocol for isolation of primary melanocytes using CnT-40 medium. Young donor tissue in general provides cells with greater proliferative capacity, but tendentially less dendricity. For the recommended thawing, passaging, and freezing protocols, please see the General Cultivation Protocol in the resources section of www.cellntec.com.

General Notes

For optimal performance, cultivate cells at 37°C with 5% CO_2.

In the prototocol below, basal keratinocytes and associated melanocytes are dislodged from the separated epidermis using vigorous pipetting, thereby minimising the number of keratinocytes that are detached. If a greater cell separation is desired, the separated epidermis can first be treated with a dissociation enzyme prior to the pipetting step - Accutase is recommended, due to its gentle action. Alternatively, trypsin / EDTA can be used.

Accutase (Cat# CnT-Accutase-100) is a mixture of proteolytic and collagenolytic enzymes isolated from crustaceans. It is free of mammalian components. Accutase is the recommended detachment enzyme due to its gentle action that leaves most surface proteins intact, and the fact that it does not require a separate reagent to stop the reaction (simply dilute with medium immediately after detachment). Accutase is heat senstive, and must be stored at 4°C. Pre-warm only the amount required to 20°C just prior to use. Exposure to higher temperatures or repeated warming / cooling cycles may lead to reduced activity.

Trypsin / EDTA is a solution of proteolytic enzymes containing Trypsin, Chymotrypsin and Elastase. Trypsin is non-defined and of porcine origin. It may vary from lot-to-lot, and must be inactivated with a separate reagent immediately after cell detachment (preferably with an animal-component free inhibitor, alternatively with FCS). Trypsin is much more aggressive than Accutase, resulting in only a very small time window between succssful detachment and irreversible cell damage. If using Trypsin, extra special care must be taken to minimise the exposure time, and ensure rapid inactivation.

Preparation

Before starting to work with the cells, ensure that the amount of medium, buffers and enzymes required have been pre-warmed at least to room temperature.

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

Isolation Procedure From Foreskin or Adult Skin

1. Place the skin directly after surgical removal into CnT-40 medium (20 mL medium in 50 mL tube). Store the tissue at 4°C until starting isolation. It is best to start within 24 hours of surgery.
2. Place the skin into a petri dish. Cut it into equally sized pieces using a scalpel blade.
3. Place the pieces of skin 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 pieces of skin overnight (~ 16 h) at 4°C in a horizontal orientation.
5. Next day transfer the all the skin pieces together with the dispase solution into a petri dish.
6. Transfer each tissue piece to a new petri dish containing CnT-40 medium to wash away excess dispase.

7. While holding the skin submerged in CnT-40 medium, gently separate the dermis (pink, opaque, gooey) from the epidermis (whitish, semi-transparent) with two pairs of curved forceps, by fixing the tissue with one pair of forceps while detaching the epidermis with the second pair. (If the separation does not occur easily, put the tissue back into the Petri dish with dispase solution for another 30 to 60 min at room temperature).
8. Prepare a centrifugation tube with CnT-40 medium containing 1 × antibiotics / antimycotics (CnT-GAB10 or CnT-ABM10); depending on tissue size, either 10 mL in a 15 mL centrifugation tube or 20 mL in a 50 mL centrifugation tube.
9. Transfer separated epidermal pieces (from step 7) with a pair of sterile forceps to the prepared centrifugation tube containing CnT-40 medium (step 8). Any larger pieces can be cut up using a scalpel to improve separation of the basal cells in the next step.
10. Pipette the medium with the epidermal pieces 40 to 50 times up and down to dislodge cells using a 10 mL serological pipette.
11. Separate the single cells by passing the suspension trough a 70 to 100 µm cell strainer.
12. Wash any remaining single cells through the cell strainer with an additional 10 ml of CnT-40 containing 1 × antibiotic / antimycotic.
13. Centrifuge the collected cells 5 min, 200 x g at room temperature.
14. Aspirate the supernatant and resuspend the cell pellet in 1 to 2 mL CnT-40 medium.
15. Count the cells.
16. Seed viable cells at a density of 4 x 10^4 cells/cm2 in culture vessels containing CnT-40 with 1 × antibiotic / antimycotic.
17. Culture the cells at 37°C and 5% CO_2.

For more information, please email out scientists directly: scientist@cellntec.com