Dermal Fibroblast Isolation

This document describes the recommended protocol for isolation of primary dermal fibroblasts using CnT-PR-F medium, an advanced new 1% serum medium that offers improved isolation efficiency and growth rate. Young donor tissue in general provides cells with greater proliferative capacity. 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.

To detach cells from the separated epidermis, Accutase is recommended. 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 foreskin directly after circumcision into CnT-PR-F medium (15 mL medium in 50 mL tube). It is best to store the tissue at 4°C until early evening before starting isolation.
2. Place the foreskin into a Petri dish. Cut it into 4 equally sized pieces using a scalpel.
3. Place the pieces of skin in a 15 mL centrifuge tube containing 10 mL of 1x dispase solution (prepared from CnT-DNP-10 with CnT-PR-F), and 2 x antibiotics / antimycotics (CnT-GAB10).
4. Incubate the skin over night (~ 16 h) at 4°C in a horizontal orientation.
5. Next day transfer the skin together with the dispase solution into a Petri dish.
6. Transfer each tissue piece to a new Petri dish containing PBS to wash away excess dispase.
7. While holding the skin submerged in PBS, 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. Cut the dermis into very small pieces using a new scalpel blade, transfer the pieces into a 50 mL centrifugation tube containing 5 mL of collagenase A (Roche #10103578001, concentration 1 mg/mL in CnT-PR-F), plus 0.5x CnT-GAB10 antibiotic/antimycotic.
9. Incubate at 37°C for 4-8 hours with occasional mixing (juvenile skin tends to be digested faster than adult).
10. Add 15 mL CnT-PR-F medium to dilute the collagenase, mix by pipetting up and down, and then pass through 70 um cell strainer (BD #08-771-2) to obtain a single-cell suspension.
11. Centrifuge the single cell suspension for 5 min, 200 x g at room temperature. Discard the supernatant and re-suspend the pellet in 1 mL CnT-PR-F.
12. Perform a cell count, and seed at approximately 4'000 cells cm^2 in CnT-PR-F.
13. Culture the cells at 37°C and 5% CO_2, with a first medium change 24-48 hours later. Then change the medium every 2-3 days.
15. Cells can be passaged using Accutase (see General Cultivation Protocol) of Trpysin (with appropriate neutralization). For maximal amplification rate, the recommended seeding density after passaging is 1'000 cells cm^2

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