

# Epidermal Keratinocyte Isolation

This document describes the recommended protocol for isolation of primary keratinocytes using CnT-Prime (CnT-PR) medium. Young donor tissue in general provides the highest cell yields, thus juvenile foreskin is used where possible. For the recommended thawing, passaging, and freezing protocols, please see the General Cultivation Protocol in the resources section of [www.cellntec.com](http://www.cellntec.com).

## General Notes

For optimal performance, cultivate cells at 37°C with 5% CO<sub>2</sub>.

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 sensitive, 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 successful 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.

## Keratinocyte Isolation from Foreskin

1. Place the foreskin directly after circumcision into CnT-PR 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), and 2 x antibiotics / antimycotics (CnT-GAB10).
4. Incubate the foreskins over night (~ 15 h) at 4°C in a horizontal orientation.
5. Next day transfer the foreskins together with the dispase solution into a Petri dish.
6. Transfer each tissue piece to a new Petri dish containing CnT-PCT medium to wash away excess dispase.
7. While holding the skin submerged in CnT-PR 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. Place a 500  $\mu$ L drop of Accutase in a new Petri dish. Lift the epidermis out of the CnT-PR medium with one pair of forceps (the tissue will collapse) and slowly transfer it onto the surface of the Accutase drop with the basal layer downward (this happens automatically). The tissue should unfold again and spread out flat on the surface of the drop, if not, remove any folds by gently agitating underneath the tissue with a second pair of curved forceps.
9. Incubate for 20 to 30 min at room temperature (cover the Petri dish with the lid to prevent evaporation).
10. Tilt the Petri dish at a  $\sim 30^\circ$  angle and add 2 mL of CnT-PR medium to the epidermis (dilutes remaining Accutase). From this point leave the dish tilted (by lifting one side onto the lid) to minimize expansion of liquid and make collection of the cells more efficient. Gently rub the epidermis on a small area of the base of the Petri-dish to gently separate single cells from the cell sheet. The medium below will become more and more turbid.
11. Use 1 to 2 mL of fresh medium to wash single cells down to the bottom of the tilted dish.
12. Transfer the single cell solution to a 15 mL centrifugation tube.
13. Repeat rubbing after adding another 2 mL of CnT-PR medium and collect cell suspension in the same 15 mL tube.
14. Centrifuge the collected cells 5 min, 180 x g at room temperature.
15. Aspirate the supernatant and resuspend the cell pellet in 2 mL CnT-PR medium.
16. Count the cells.
17. Seed viable cells initially at a density of  $2 \times 10^4$  cells /  $\text{cm}^2$  in culture vessels (density may be reduced to 4'000 cells  $\text{cm}^2$  in subsequent passages).
18. 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%  $\text{CO}_2$ . Change medium after 24 hours to remove unattached cells, and then after 3 days (avoid too frequent medium changes initially when cells are at lower densities, as it also removes beneficial factors secreted by the cells).

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