Corneal Epithelium Progenitors, Human

Caution: *This material is of human origin and thus potentially hazardous. It has tested negative for HIV 1, Hepatitis B, and Hepatitis C, but testing is not 100% accurate. Treat this material as infectious, and use appropriate biocontainment, protective equipment, and other precautions to prevent accidental exposure.*

CATALOG NUMBER:	HCEP-05	**QUANTITY:**	1 kit containing Cells and Media

PRODUCT DESCRIPTION: Normal human corneal progenitor cells cryo-preserved cells frozen in CELLnTEC CRYO-Defined, Animal Component Free Freezing medium.. Primary cells supplied with one 500 ml kit of medium (basal medium with separate frozen supplements)

SPECIES/SOURCE: Human/Single Donor Cornea

FORMAT: 1 Kit consisting of

1. 1 x HCEP-C: cryotube containing >5 x 10^5 cells
2. 1 x CnT-20: 500 ml kit of #CnT-20 medium

CULTURE MEDIUM: PCT Corneal Epithelium Medium, Defined (CnT-20) or PCT Corneal Epithelium Medium, Low BPE (CnT-50) for growth. Corneal Epithelium Medium (CnT-30) for differentiation experiments.

CULTIVATION: Following thawing, we recommend seeding cells at 4.0 x 10^3 cells per cm^2 (e.g. one vial of cells into 5 x 25 cm^2 flasks). Lower seeding densities are possible but time to confluence is extended. See the resources page of www.cellntec.com for full protocols.

AVERAGE TIME TO CONFLUENCY: 6-8 days (depending on temperature, protocol and seeding density)

FURTHER GROWTH: Depending on seeding density and culture protocol, these cultures have been found to deliver more than 15 population doublings when grown in a PCT medium.

QUALITY CONTROL: Tested negative for Hepatitis B, Hepatitis C and HIV-1. Free of bacteria, fungi and mycoplasma contamination

STORAGE: Cryotube: Supplied frozen on dry ice. Immediately upon arrival transfer the vial to liquid N_2 for storage until ready to use

Medium: Refer to medium labels for specific instructions

Corneal Epithelium Progenitors, Human: PROTOCOL

Below are various protocols for HCEP cells. These cells may be cultured in either CELLnTEC's PCT media, namely CnT-20 (defined) or CnT-50 (low-BPE). These media are referred to below as "CnT-medium"

Important Note: Primary cells differ from immortalized cell lines in a number of ways. Primary cells require careful attention to protocol detail to maximize thawing survival and subsequent normal cell growth. The following protocol will allow successful growth of difficult to grow primary cells. Please first review prior to the thaw of the cells. If you have questions or want to deviate from the protocol, please contact CELLnTEC at scientist@cellntec.com

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Immediately upon delivery

Remove the vial from the shipping container, and check that it is still frozen. Transfer frozen vial to liquid nitrogen until you are ready to thaw and begin culture.

Thawing cells

1) Thaw cells rapidly until just melted. CAUTION: If thawing in a 37 ºC waterbath, be careful to remove the vial while there is still a small amount of ice left. Do not allow the cells to remain in their freezing medium or cell viability will be reduced. Immediately proceed to the next step.
2) Resuspend pellet in 5 mL CnT-medium.
3) Seed the cells at 4 x 10^3 cells per cm2 (e.g. 5 x 10^5 cells into five, 25 cm^2 culture flasks) with a final volume of 5 mL per 25 cm^2 culture flask (Note: lower amounts, e.g. 2.0 x 10^3 cells per cm^2 can also be seeded but will take longer until confluence)

IMPORTANT: Cells need to be removed from the detrimental effect of DMSO by change the medium the next day.

Growth

1) Let the cells grow at 35 °C and 5% CO2 changing the medium every 3rd day.
2) They should approach confluence in approximately 6-10 days.
3) Passage the cells prior to confluence (80% is best as differentiation may initiate with over confluent cultures).

Passaging

1) Aspirate the medium. Add 2 mL of 1x rProtease and incubate until all cells are detached (ca. 10 min. at 35 °C).
2) Add 5 mL CnT-medium to the detached cells and resuspend 2-3 times vigorously.
3) Spin the cells at 160 g for 5 min
4) Aspirate supernatant and resuspend the pellet in 5 mL CnT-medium .
5) Count cells and seed at the appropriate density with a final volume of 5 mL per 25 cm^2 culture flask.

Seeding

1) Until you become experienced with the cells, we recommend seeding $8x10^3$ cells/cm^2 back into the same flask after rinsing it with another 5 mL of CnT medium, seeding $8x10^3$ cells/cm^2 into a new flask and seeding $4x10^3$ cells/cm^2 into a new flask.
2) Recommended seeding density: $4x10^3$ cells/cm^2 in culture flasks in 5 mL CnT-medium /25cm^2 normally allows weekly passage however good growth is also observed at lower seeding densities.

Medium change

1) Aspirate all medium and replace with fresh CnT-medium.
2) Change medium 2 days after seeding, then every 3 days.

Freezing

1) Treat subconfluent monolayers with rProtease as above.
2) Count cells - place the cells on ice while counting.
3) Adjust the cell concentration to two times the number of cells you want to freeze per mL with cold CnT-medium (e.g. for a final concentration of 1×10^6 cells/mL and one mL per cryotube, we adjust the concentration at this step to 2×10^6 cells/mL).
4) Add drop-wise the same amount of cold 2 x freezing medium, while gently swirling the tube (final concentration 1×10^6 cells/mL).
5) Immediately add 1 mL cell suspension into labeled cryotubes.
6) Immediately transfer tubes to a NALGENE® Cryo 1°C freezing container (#5100-0001).
7) Immediately place at –80°C, leave at least overnight.
8) Transfer tubes to liquid nitrogen for long-term storage.

Solutions

rProtease: Tryple™ Select (12563-011, Invitrogen)

For research use only. Not for use in humans, animals, or diagnostics.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.