

## Product Information

# HIGH QUALITY LOW PASSAGE PRIMARY SKIN CELL CULTURES

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Fibroblasts, Keratinocytes and Melanocytes derived from skin and scar/keloid tissue (*various anatomical regions available; juvenile, adult and aging*), provided in T75 cultured flasks. 2ml cryopreserved vials are also available.

> 500,000 viable or proliferating cells

### Product Line

- Normal Human Dermal Fibroblasts (**NF**)
- Keloid Fibroblasts (**KF**)
- Hypertrophic Scar-derived Fibroblasts (**HSF**)
- Fibroblast isolated from Normal Scar (**NSCF**)
- Fibroblasts isolated from normal skin adjacent to Keloid Fibroblasts (**nsKF**) or Hypertrophic Scars (**nsHSF**)
- Fibroblasts isolated from Aging Skin (**asF**)
- Normal Human Keratinocytes (**NK**)
- Keloid-derived Keratinocytes (**KK**)
- Hypertrophic Scar-derived Keratinocytes (**HSK**)
- Keratinocytes isolated from Normal Scar (**NSCK**)
- Keratinocytes isolated from normal skin adjacent to Keloid Fibroblasts (**nsKK**) or Hypertrophic Scars (**nsHSK**)

### **Cell Culture Description**

Using Explant Technique as the primary cell culture method, the high quality low passage cell strains are derived at CellResearch's cell culture facility from skin and scar/keloid tissue obtained from surgical procedures. Each strain is obtained from one individual and isolated according to referenced procedures. Proliferating cell cultures are made from cryopreserved cells that have been thawed and cultured for three days at CellResearch's cell culture facility. Cells are not pooled or transformed.

## Proliferating Capacity

CellResearch's cell cultures are derived with the use of careful methods, from skin and scar/keloid tissue (in vivo state). They are not transformed and have a limited lifespan in vitro. All strains are tested for their proliferative capacity in CellResearch's cell culture facility.

## Quality Test

All cell cultures from CellResearch are subjected to stringent quality tests before shipment. Comprehensive testing include HIV-1 PCR, HBV DNA PCR and HCV RNA PCR. Certificate of Analysis (CoA) will accompany shipments.

### Maintenance of Cryopreserved cells

Upon receipt of delivery, the vials with the cryopreserved cells must be taken out of the dry ice container immediately and;

- be transferred to a storage facility with liquid nitrogen (-196°C), or
- thawed and put each vial in a T75 tissue culture flask in DMEM/10%FCS (Fibroblast) or Serum-Free Medium (Keratinocyte)

### Maintenance of Proliferating cells

Upon receipt of delivery,

- Check the proliferating culture for signs of damage during dispatch (e.g. atypical morphology). The bottles should show many cell "islands". Determine the cell density by estimating the "confluence %".
- Place the closed culture flask in a 37°C, humidified incubator with 5% CO<sub>2</sub>.
- Prepare medium.
- Wipe the culture flask with 70% ethanol and wait until the alcohol has evaporated before opening the culture flask in a laminar airflow cabinet. Remove the medium with a pipette without touching the cell monolayer. Replace the medium with 10 ml fresh medium. In order to prevent contamination make sure that there are no traces of medium left on the inner / outer part of the neck of the culture flask.
- Place the filled cell culture flask in a 37°C, humidified incubator with 5% CO<sub>2</sub>. Close the screw lids on the culture flask by half a turn only to allow gas exchanges to take place.
- The cells are ready for sub-culturing after 12 to 48 hours.

## Warning Note (Use of biological material)

CellResearch's cell cultures are of human origin and while every cryopreserved cells have been tested as per our quality test, no diagnostics tests can ensure the total absence of infectious agents. All cells of human origin should be treated as potential pathogens.



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FOR RESEARCH USE ONLY. NOT TO BE USED FOR DIAGNOSTICS OR THERAPEUTIC PURPOSES.

### Ordering and Technical Information

CellResearch Corporation Pte Ltd  
#03-09, BIK MD-11, Clinical Research Centre  
10 Medical Drive. Singapore 117597

Tel: (+65) 6874-3357  
Fax: (+65) 6722-4261  
Email: [info@cellresearchcorp.com](mailto:info@cellresearchcorp.com)  
Web: [www.cellresearchcorp.com](http://www.cellresearchcorp.com)





## Human Epidermal Keratinocyte Cell Systems

### Instructions for Use

#### Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – If there is dry ice left in the package, place cryovials immediately into liquid nitrogen. If no dry ice is left in the package, thaw and use them immediately.
3. For proliferating cells – Swab down the flask of proliferating cells with 70% ethanol or isopropanol, then place the flask in 37°C, 5% CO<sub>2</sub>, humidified incubator and allow equilibrating for three to four hours. After cells have equilibrated, remove shipping medium from the flask and replace with fresh medium.

#### Cell Culture Medium and Reagents:

EpiLife Basal Medium (Cascade Biologics Cat# M-EPI-500-CA): 500ml/bottle

EpiLife Defined Growth Supplement (EDGS): 5ml/vial

Prepare EpiLife Growth Medium: EpiLife supplemented with 1% EDGS and 1% Antibiotics/Antimycotics

1x Trypsin-EDTA solution (from Trypsin-EDTA (10X), 0.5% Trypsin with EDTA Cat#15400-054, Invitrogen Corporation)

#### Cell Culture Process

1. The recommended seeding density for Human Epidermal Keratinocytes is 3,300 cells/cm<sup>2</sup> or 250,000 cells/T75 tissue culture flask. Cells will reach 70%-80% confluent in 5-7 days.
2. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, and then retighten.
3. Quickly thaw the cryovial in a 37°C water bath being careful not to submerge the entire vial.
4. Resuspend the cells in the cryovial and using a micropipette, dispense cells into the culture vessels set up earlier. Gently rock the culture vessel to evenly distribute the cells and return to the incubator.
5. Centrifugation should not be performed to remove cells from cryoprotectant cocktail. This action is more damaging than the effects of DMSO residue in the culture.

### Sub-culture of Human Epidermal Keratinocytes

The following instructions are for a 75 cm<sup>2</sup> flask. Adjust all volumes accordingly for other size flasks.

#### Preparation for subculturing the first flask:

1. Subculture the cells when they are 70% to 80% confluent and contain many mitotic figures throughout the flask.

**Important: Do not let cells confluent more than 80%. At this stage, cells can be growth-arrest and do not proliferate well in next passage**

2. For each 75 cm<sup>2</sup> of cells to be subcultured:

Add 5 ml of warm 1X Trypsin/EDTA in each flask.

Incubate in incubator for less than 5min.

Shake gently to detach cells from bottom of the flasks.

Transfer to centrifuge tube.

Pipette up and down vigorously to break cell clump.

Add in the tube 4ml DMEM/10%FCS to quench trypsin action.

Centrifuge at 1200 rpm for 5 min.

Suspend cell pellet in EpiLife Growth Medium for further experiment or cryopreservation

3. Subculture 1-3 flasks at a time.

4. Cryo-preserve cells in EpiLife Growth Medium/10%DMSO. Recommend cell density of 500,000-2,000,000 cells/2ml cryovial.

**Note:** *It is recommended not to go beyond Passage 4.*

## Frequently Asked Questions (FAQ)

### Technical

#### 1. Can I freeze CellResearch's cell strains?

It is not advisable to freeze the cell strains as this may lead to a degradation of the cells and their proliferating potential.

#### 2. What techniques are you using to isolate the cell strains?

Explant Technique is used in the isolation of the cell strains.

#### 3. Are CellResearch's cells strains derived from a single individual or are they pooled from several donors?

CellResearch's cell strains are derived from single donors and are not pooled.

#### 4. Which anatomical regions are the cell strains derived from?

We have cell strains from various anatomical regions such as earlobe, groin, wrist, leg, etc. Please consult with our sales specialists.

#### 5. Are the Keloid fibroblasts considered cancerous?

The Keloid fibroblasts are not cancerous, but may be considered benign tumor. This is because there are some cancerous characteristics, such as fast proliferation, invasiveness, fast migration, apoptotic genes are down regulated and anti-apoptotic genes are up regulated, etc.

#### 6. Which cells produce more collagen?

Keloid fibroblasts produce approximately 20 times more collagen than normal dermal fibroblasts while aging fibroblasts produce less collagen than young skin fibroblasts.

#### 7. Which cells are more elastic?

Keloid and Hypertrophic Scar cells are less elastic than normal skin cells.

#### 8. Are the cells tested against potential infectious hazards?

The cells are analyzed for HIV, HBV and HCV. However, note that CellResearch's cell cultures are of human origin and while every effort has been taken to test the cells, no diagnostics tests can ensure the total absence of infectious agents. All cells of human origin should be treated as potential pathogens.

### General

## 1. **Why should I use CellResearch's cell strains?**

CellResearch's cell strains are obtained from single donors and are not pooled. We have strains derived from multiple locations of the body. Besides the normal human skins, we also have cell strains derived from Keloid and Hypertrophic Scars. For comparative research, fibroblasts isolated from normal skin adjacent to the Keloid or Hypertrophic Scar is also available.

## 2. **How are the cells supplied?**

As proliferating cells in CF, unless specified. Cryopreserved vials are also available upon request.

If your questions have not yet been answered, please contact our [Technical Specialists](#).

They will be most happy to clarify your queries.