EZxpand™ Dermal Fibroblast Culture Kit

Product Code: CCK027

1. Product Description

HiMedia’s EZxpand™ Culture Kits are complete and ready-to-use kits designed for hassle-free in vitro culturing of different primary cells and mesenchymal stem cells. These kits contain cryopreserved or proliferating primary cells / stem cells, expansion medium and antibiotic-antimycotic solution.

CCK027 is EZxpand™ Dermal Fibroblast Culture Kit designed for in vitro culturing of human adult dermal fibroblast cells.

2. Kit Contents

<table>
<thead>
<tr>
<th>Code</th>
<th>Content</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK027 (A)</td>
<td>HiFi™ Human Adult Dermal Fibroblast (HADF)</td>
<td>1 vial (0.5 million cells/vial)</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td></td>
</tr>
<tr>
<td>CCK027 (A)</td>
<td>HiFi™ Human Adult Dermal Fibroblast (HADF)</td>
<td>1 T25cm² flask</td>
</tr>
<tr>
<td>CCK027 (B)</td>
<td>HiFibroXL™ Fibroblast Expansion Medium, Reduced Serum (Basal)</td>
<td>500ml</td>
</tr>
<tr>
<td>CCK027 (C)</td>
<td>HiFibroXL™ Fibroblast Expansion Supplement</td>
<td>12ml</td>
</tr>
<tr>
<td>CCK027 (D)</td>
<td>Gentamicin-Amphoterecin B Solution 1000X</td>
<td>0.5ml</td>
</tr>
</tbody>
</table>

3. Description of Kit Contents

**Part A: HiFi™ Human Adult Dermal Fibroblast (HADF)**

Source: Human

Tissue: Skin (Dermis)

Type of cells: Fibroblast

Growth characteristics: Adherent

HiFi™ Human Adult Dermal Fibroblast (HADF) are isolated from excess human skin remaining after cosmetic surgery. HADF are Passage 2 cells supplied frozen with density of not less than 0.5 x 10^6 cells per vial or as proliferating cells in a T-25 culture flask. Each lot of cells is from a single donor and undergoes growth kinetic studies and cell marker analysis by flow cytometry. Cells are maintained in antibiotic free conditions prior to supply.

**Part B: HiFibroXL™ Fibroblast Expansion Medium, Reduced Serum**

HiFibroXL™ Fibroblast Expansion Medium is designed for in vitro cultivation and expansion of human dermal fibroblasts. This medium enables robust growth and expansion of dermal fibroblast.

The medium contains inorganic and organic salts, amino acids, vitamins, growth factors, nutrients and sodium bicarbonate. It does not contain antibiotics and antifungal agents.

**Part C: HiFibroXL™ Fibroblast Expansion Supplement**

It is a proprietary formulation containing growth factors and nutrients necessary for growth of fibroblasts.

**Part D: Gentamicin-Amphoterecin B Solution 1000X**

It is a sterile filtered solution formulated to contain 30mg/ml Gentamicin and 25ug/ml Amphoterecin B in sterile tissue culture grade water.

Gentamicin is broad spectrum bactericidal agent of aminoglycoside group effective against Gram positive and Gram negative bacteria. It interferes with protein synthesis by binding to 30S subunit of ribosome. Amphotericin B is an antifungal agent effective against fungi and yeasts. It interferes with the cell membrane permeability of sensitive fungi and yeasts by forming channels and causing leakage of low molecular weight substances.

Gentamicin - Amphoterecin B solution 1000X is specially designed to minimize the chances of contamination in primary cell cultures.
4. Product Warranty

HiFi™ cells are performance tested using HiMedia media and reagents. We recommend using the product codes mentioned in this sheet for optimum performance and reliability.

5. Materials required but not provided in the kit

- Dulbecco’s Phosphate Buffered Saline (DPBS) (TL1006)
- Trypsin/EDTA Solution 1X (TCL007)
- Trypan Blue 0.5% solution (TCL005)
- T25 or T75 cell culture treated flask
- Other consumables

6. Directions for Use

Users are advised to review entire procedure before starting the culture

6.1. Storage and Handling of cells on Receipt

6.1.1 Cryopreserved cells

Cryopreserved cells are supplied in liquid nitrogen dry vapour shipper at -150°C or below. Upon receipt, immediately transfer the vial to the vapor phase of liquid nitrogen tank. Store it in the tank until further use.

6.1.2 Proliferating cells

1. Proliferating cells are supplied in a T-25 flask at room temperature. The flasks are filled to capacity with transport medium. The neck is sealed with parafilm and each flask is individually packed in a plastic bag to contain leakage if any.
2. Handling of flask should be done in an area designated for use with human origin material. Proper precautions and biological containment should be taken while handling human cells due to their potential bio-hazardous nature.
3. Always wear protective clothing, gloves and work behind a protective screen while handling human cells.
4. Check the bag for evidence of any leakage during transport. If the flasks have leaked or broken, discard the entire content following guidelines prescribed for bio-hazardous waste.
5. If the flask is intact and there is no evidence of leakage, remove the flask from the plastic bag.

6. Transfer the flask to an incubator at 37°C and 5% CO₂ for 3-4 hours. This is required to help floating cells reattach to the surface.

6.2. Preparation of complete medium

1. On receipt, store the medium at 2 - 8°C and growth supplement and Gentamicin-Amphotericin B solution at -20°C in dark until use.
2. Thaw fibroblast growth supplement (CCK027(C)) overnight at 2-8°C.
   Note: Precipitates in Part C after thawing is normal. Precipitates will not affect the performance of the medium.
3. Disinfect the external surface of the bottles of Part B and Part C by spraying with isopropyl alcohol before placing in a biosafety hood.
4. Transfer the entire content of Part C to basal medium (Part B) under aseptic condition.
5. Aseptically add 0.5ml of Gentamicin-Amphotericin B solution (Part D) to 500ml of complete medium.
6. Tightly cap the bottle and swirl gently to ensure proper mixing.

Note: Do not mix vigorously. Doing so will cause formation of foam

6.3. Initiating cultures from cryopreserved cells

1. Add appropriate volume of complete medium into the culture vessel(s) (5ml for a T25 flask and 15ml for a T75 flask).
2. Carefully remove the vial from liquid nitrogen tank and thaw quickly by placing the frozen vial of cells in a water bath at 37°C.
   Caution: Wear appropriate personal protective equipment to collect the cells from liquid nitrogen storage.
3. Hold the frozen vial of cells in the water bath with lower half immersed in water.
   Note: Avoid getting the water up to the cap of the vial to decrease the chance of contamination.
4. Keep swirling the vial until the frozen clump inside it thaws completely. Thawing of cells should not take more than 60-90 seconds.
5. Swab the vial thoroughly with 70% isopropanol and open it in a laminar hood.
6. Pipette gently to get a homogenous mixture of cells.
   Note: If you want to seed with a specific seeding density, follow point 8 or else follow point 7.
7. Pipette the entire volume of cells directly into the flask(s) containing medium.
8. Set aside an aliquot of 40-50µl of cells for determination of cell density and viability if you want to seed cells with a specific seeding density. Determine the viability and density of...
cells per ml using trypan blue and a hemocytometer. Seed flask(s) with the desired seeding density (recommended seeding density is 5000 to 10000 cells per cm²).

Note: Refer Table 1 for more details.

**Table 1**:  

<table>
<thead>
<tr>
<th>Flask</th>
<th>Seeding density</th>
<th>Total number of cells per flask</th>
<th>No. of flasks seeded with 1 vial</th>
<th>Volume of medium (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T25</td>
<td>5000 cells/cm²</td>
<td>0.125 x 10⁶</td>
<td>4</td>
<td>5 - 7</td>
</tr>
<tr>
<td></td>
<td>10000 cells/cm²</td>
<td>0.25 x 10⁶</td>
<td>2</td>
<td>5 - 7</td>
</tr>
<tr>
<td>T75</td>
<td>5000 cells/cm²</td>
<td>0.375 x 10⁶</td>
<td>1</td>
<td>10 - 15</td>
</tr>
</tbody>
</table>

*These are recommended seeding densities from literature and our own studies. Higher seeding densities do not cause any harm to the cells and lower the required population doublings per passage. Lower seeding densities may cause cells to lose viability, detach during culture and in general take larger population doublings to reach confluence.

9. Rock the flask(s) gently to ensure proper mixing of cell suspension and medium and to ensure uniform distribution of cells. Incubate in a humidified incubator at 37°C saturated with 5% CO₂.
10. Replenish the culture vessel with fresh medium and incubate further.
11. Monitor cells microscopically every day.
12. Give medium change medium every two to three days. Subculture once cells reach 70-80% confluence.

**6.4. Culturing from proliferating cell flasks**

1. After 3-4 hour incubation period, remove the flasks from the incubator. Disinfect it by spraying with isopropyl alcohol and transfer it to the Biosafety hood.
2. Keep the flask in standing position. Remove the parafilm.
3. Remove the cap and aseptically aspirate entire medium from the flask and discard. Aspirate any medium that may be on the threads of the cap or the neck of the flask.
4. Replenish flask with appropriate volume of complete medium.
5. Replace the cap and transfer the flask to humidified incubator at 37°C saturated with 5% CO₂.
6. Monitor cells microscopically every day.
7. We recommend not disturbing the culture for 48-72 hours after the culture has been initiated.
8. Give medium change every two to three days. Subculture once cells reach 70-80% confluence.

**6.5. Sub-culture**

A confluent T25 flask of HADF yields 1.0 to 1.5 x 10⁶ cells and a T75 flask yield approximately 2.0 to 3.0 x 10⁶ cells.

Sub-culturing ratios can vary from 1:2-1:5. HADF can be sub-cultured at a seeding density of 5000-10,000 cells/ cm² as per Table 1.
1. Aseptically aspirate entire medium without disturbing the monolayer and discard.
2. Wash the cells with appropriate volume of DPBS (2-3 ml for a T25 flask, 5-7ml for a T75 flask) to remove residual medium.
3. Aseptically aspirate off the DPBS and discard.
4. Aseptically add appropriate volume of pre-warmed Trypsin-EDTA solution to the flask. (500µl for a T25 flask, 1ml for a T75 flask). Gently rock the flask to ensure complete coverage of the Trypsin-EDTA solution over the cells.
5. Incubate the flask in the incubator for 3-5min.
6. Microscopically monitor the flask. When the cells start rounding up, gently tap the flask to ensure complete detachment of cells.
7. Neutralize action of Trypsin by addition of complete medium. Pipette gently to get a homogenous mixture of cells.
8. Count cells and seed fresh flasks at recommended seeding density.
9. Incubate in a humidified incubator at 37°C saturated with 5% CO₂.
10. Monitor cells microscopically every day.
11. Give medium change every two to three days. Subculture once cells reach 70-80% confluence.

**7. Quality Control**

**Cells**

**Growth Performance Assay**

No. of viable cells/vial: NLT 500,000 cells/vial  
Percentage viability: NLT 75%  
Total number of population doublings: NLT 15

**Marker Analysis Assay**

**By Flow Cytometry**

Positive Marker (>95% events)  
CD90: Positive  
Vimentin: Positive
Sterility Assay
Mycoplasma: Not detected
Bacteria, Fungi and Anaerobes: Not detected
HIV virus: Not detected
Hepatitis B Virus: Not detected
Hepatitis C Virus: Not detected

Expansion Medium
Appearance
Part B: Orangish red coloured clear solution
Part C: Pale yellow coloured clear solution

pH
7.00 - 7.60

Osmolality in mOsm/Kg H2O
280.00 - 320.00

Sterility
No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

Cultural Response
The medium is tested for optimal cell growth and proliferation of fibroblasts.

8. Storage and shelf life:

<table>
<thead>
<tr>
<th>Code</th>
<th>Content</th>
<th>Storage</th>
<th>Shelf life</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK027 (B)</td>
<td>HiFibroXL™ Fibroblast Expansion Medium, Reduced Serum</td>
<td>2-8°C</td>
<td>Refer expiry date given on the product label</td>
</tr>
<tr>
<td>CCK027 (C)</td>
<td>HiFibroXL™ Fibroblast Expansion Supplement</td>
<td>-20°C</td>
<td></td>
</tr>
<tr>
<td>CCK027 (D)</td>
<td>Gentamicin-Amphotericin B Solution 1000X</td>
<td>-20°C</td>
<td></td>
</tr>
<tr>
<td>Complete medium</td>
<td></td>
<td>2-8°C</td>
<td>6 weeks</td>
</tr>
</tbody>
</table>

Note: Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement.

9. Related Products

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Code</th>
<th>Packing</th>
</tr>
</thead>
<tbody>
<tr>
<td>EZXpand™ Human Umbilical Vein Cell Kit</td>
<td>CCK026-0.5</td>
<td>0.5 x 10⁶ cells/vial</td>
</tr>
<tr>
<td>Endothelial Cell Culture Kit</td>
<td>CCK026-T25</td>
<td>1 T25 cm² flask</td>
</tr>
</tbody>
</table>

Warning
This product is intended for research use only. Not for animal, human therapeutic or diagnostic use. Product contains Human origin material and should be treated as potentially infectious. Even if cells provided have been screened for viral and bacterial pathogens, Human cells may harbor other known or unknown agents which might pose a health hazard. Universal handling precautions applicable to biological samples must be applied as recommended in the CDC-NIH manual.

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