EZXpand™ Umbilical Vein Endothelial Cell Culture Kit

Product Code: CCK026

1. Product Description
HiMedia’s EZXpand™ Culture Kits offer a 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.

CCK026 is EZXpand™ Human Umbilical Vein Endothelial Cell Culture Kit designed for in vitro culturing of human umbilical vein endothelial cells.

2. Kit Contents

<table>
<thead>
<tr>
<th>Code</th>
<th>Content</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK026 (A)</td>
<td>HiFi™ Human Umbilical Vein Endothelial Cells (HUVEC)</td>
<td>1 vial (0.5million cells/ vial)</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK026 (A)</td>
<td>HiFi™ Human Umbilical Vein Endothelial Cells (HUVEC)</td>
<td>1 T25cm² flask</td>
</tr>
<tr>
<td>CCK026 (B)</td>
<td>HiEndoXL™ Endothelial Cell Expansion Medium, Reduced Serum (Basal)</td>
<td>500ml</td>
</tr>
<tr>
<td>CCK026 (C)</td>
<td>HiEndoXL™ Endothelial Cell Expansion Supplement</td>
<td>14.3ml</td>
</tr>
<tr>
<td>CCK026 (D)</td>
<td>Gentamicin-Amphotericin B Solution 1000X</td>
<td>0.5ml</td>
</tr>
</tbody>
</table>

3. Description of Kit Contents

Part A: HiFi™ Human Umbilical Vein Endothelial Cells (HUVEC)
Source: Human
Tissue: Umbilical cord vein endothelium
Type of cells: Endothelial
Growth characteristics: Adherent

HiFi™ Human Umbilical Cord Vein Endothelial Cells (HUVEC) are isolated from human umbilical cords collected post partum. HUVEC are Passage 2 cells supplied frozen with density of not less than 0.5 x 10⁶ cells per vial or as proliferating cells in a T-25 or a T-75 culture flask. Each lot of cells is from a single donor and undergoes growth kinetic studies and marker analysis. Cells are maintained in antibiotic free conditions prior to supply.

Part B: HiEndoXL™ Endothelial Cell Expansion Medium, Reduced Serum

HiEndoXL™ Endothelial Cell Expansion Medium is a reduced serum medium used for in vitro cultivation and expansion of human umbilical vein endothelial cells (HUVEC). This medium enables superior growth of endothelial cells as compared to serum supplemented classical medium. The medium is supplied in two parts, the basal medium and growth supplement. Basal medium contains inorganic and organic salts, amino acids, vitamins, growth factors, nutrients and sodium bicarbonate. It does not contain antibiotics and antymycotics.

Part C: HiEndoXL™ Endothelial Cell Expansion Supplement

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

HiFiTM 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

- Fetal bovine serum (FBS) (RM1112 / RM10432)
- Dulbecco’s Phosphate Buffered Saline (DPBS) (TL1006)
- Trypsin/EDTA Solution 1X (TCL007)
- Trypan Blue 0.5% solution (TCL005)
- 0.5% Gelatin solution in DPBS (TCL109)
- 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 basal medium at 2 - 8°C and growth supplement and Gentamicin-Amphotericin B solution at -20°C in dark until use.

2. Thaw endothelial cell growth supplement (CCK026(C)) overnight at 2-8°C.

3. Note: Precipitates in Part C after thawing is normal. Precipitates will not affect the performance of the medium.

4. Disinfect the external surface of the bottles of Part B and Part C by spraying with isopropyl alcohol before placing in a biosafety hood.

5. Transfer the entire content of Part C to basal medium (Part B) under aseptic condition.

6. Aseptically add 0.5ml of Gentamicin-Amphotericin B solution (Part D) to 500ml of complete medium.

7. 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 growth 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>
<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. Change the 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 the flask 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 fresh 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. Change the medium every two to three days. Subculture once cells reach 70-80% confluence.

6.5. Sub-culture

A confluent T25 flask of HUVEC yields 2.0 to 3.0 x 10⁶ cells and a T75 flask yield approximately 8.0 to 10.0 x 10⁶ cells.

Sub-culturing ratios can vary from 1:2-1:5. HUVEC 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 containing FBS. 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. Change the medium every two to three days. Subculture once cells reach 70-80% confluence.
6.6. Gelatin coating of culture vessel:

Note: Coating of culture vessel with gelatin enhances attachment of HUVEC to the vessel surface.

1. Aseptically add 0.5% gelatin solution (TCL109) to the culture vessel. Refer Table 2 for recommended volumes of gelatin solution for different culture vessels.
2. Incubate overnight at 37°C incubator.
3. After incubation, aseptically aspirate the gelatin solution with the help of pipette and again incubate the culture vessel at 37°C incubator until it is dried.
4. Use this gelatin-coated culture vessel for culturing.

Note: For uniform coating, make sure that the incubator is properly leveled.

Table 2: Recommended volumes of Gelatin solution for different culture vessels

<table>
<thead>
<tr>
<th>Culture vessel</th>
<th>Volume per well</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well plate</td>
<td>75 µl</td>
</tr>
<tr>
<td>48-well plate</td>
<td>150 µl</td>
</tr>
<tr>
<td>24-well plate</td>
<td>300 µl</td>
</tr>
<tr>
<td>12 well plate</td>
<td>500 µl</td>
</tr>
<tr>
<td>6 well plate</td>
<td>1 ml</td>
</tr>
<tr>
<td>T25 flask</td>
<td>5 ml</td>
</tr>
<tr>
<td>T75 flask</td>
<td>10 ml</td>
</tr>
</tbody>
</table>

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

a. By Immunocytochemistry
von Willebrand Factor (vWF): Positive
Smooth muscle α-actin: Negative

b. By Flow Cytometry∗

Positive Marker (>95% events)
CD31

This data has been generated on 8 parameter 3 laser Partec CyFlow® Cube 8 Flow Cytometer and is specific for representative batch of HUVEC. For batch specific information, refer Certificate of Analysis (COA) of given batch on the Website www.himedialabs.com

Sterility Assay
Mycoplasma: Not detected
Bacteria, Fungi and Yeast: Not detected

Virus Testing
HIV: 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 endothelial cells.
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>CCK026 (B)</td>
<td>HiEndoXL™ Endothelial Cell Expansion Medium, Reduced Serum</td>
<td>2-8°C</td>
<td>Refer expiry date given on the product label</td>
</tr>
<tr>
<td>CCK026 (C)</td>
<td>HiEndoXL™ Endothelial Cell Expansion Supplement</td>
<td>-20°C</td>
<td></td>
</tr>
<tr>
<td>CCK026 (D)</td>
<td>Gentamicin-Amphotericin B Solution 1000X</td>
<td>-20°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Complete medium</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 Adult Dermal Fibroblast Culture Kit</td>
<td>CCK027-0.5</td>
<td>0.5 x 10^6 cells/vial</td>
</tr>
<tr>
<td></td>
<td>CCK027-T25</td>
<td>1 T25cm² 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 the 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.