Cellworks
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PRODUCT INFORMATION

Human Umbilical Vein Endothelial Cells (HUVEC) (angiogenesis co-culture validated)

Product Code: ZHC-2102

Validation

These cells have been shown to form tubules in our angiogenesis assay co-culture with Cellworks Human Dermal Fibroblasts (HDF) (angiogenesis co-culture validated) (Product Code: ZHC-5102) using Cellworks Angiogenesis Growth Medium (Product Code: ZHA-1970).

Presentation

Each cryovial of HUVEC contains a minimum of 500,000 cells from multiple donors, guaranteed to be 70% viable after thawing. Cells are cryopreserved at passage 2 in a cryoprotectant-containing medium.

Recommended culture medium

Human Large Vessel Endothelial Cell Growth Medium Package (Product Code: ZHM-2953)

Contains: Human Large Vessel Endothelial Cell Basal Medium, 500ml (KC1015)
Human Large Vessel Endothelial Cell Growth Supplement, 10ml (KC1014)
Antibiotic/Antimycotic Supplement, 0.5ml (KC1019)

For angiogenesis co-culture assays, we recommend culturing these cells with Cellworks Human Dermal Fibroblasts (ZHC-5102) in Cellworks Angiogenesis Growth Medium (ZHA-1970). We recommend not culturing these cells beyond passage 5 prior to use in an angiogenesis assay.

Recommended seeding density

2,500 viable cells per cm^2 (see overleaf for brief guidelines on initiating and maintaining proliferating cultures from cryopreserved cells)

Proliferation capacity

Normal human cells have a limited life span *in vitro*. When these cells are cultured using our recommended reagents and procedures, Cellworks guarantees 10 population doublings.

Delivery and storage

Cryopreserved cells require **immediate attention** upon receipt. These cells will arrive frozen in cryovials on dry ice and must be either seeded immediately or transferred to liquid or vapour phase nitrogen storage (-135°C to -196°C). Continued storage on dry ice or at -80°C is **not** appropriate.

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Brief guidelines for culture of Cellworks cells

1. Prepare a bottle of Human Large Vessel Endothelial Cell Growth Medium according to the Cellworks instructions
2. Pre-equilibrate 1x 15ml in 1x 75cm^2 cell culture flask or 3x 5ml in 3x 25cm^2 cell culture flasks in a humidified incubator (37°C, 5% CO_2)
   N.B. *A minimum of 15ml culture medium must be used to dilute the cryoprotectant*
3. Prepare a water bath at 37°C
4. Obtain the cryovial of cells from nitrogen storage and transfer to dry ice
5. Gently agitate the cryovial in the 37°C water bath, ensuring that the cap is not submerged, until the cell suspension is just thawed
6. Immediately wipe the cryovial with alcohol and place in a sterile laminar flow hood
7. Use a pipette to gently mix the cell suspension
8. Aliquot 20µl cell suspension and dilute 1:1 with 20µl Trypan blue
9. Count the cells using a haemocytometer (or equivalent) to determine the number of viable cells per ml
10. Inoculate the prepared flask(s), diluting the cells to a concentration of at least 1.25 x 10^4 viable cells per ml (equivalent to 2500 viable cells per cm^2), by dispensing the cell suspension in an arc on the surface of the medium
11. Gently agitate the flask(s) to evenly distribute the cells
12. Place the flask(s) in a 37°C, 5% CO_2 humidified incubator and allow cells to adhere
    N.B. *For best results, do not disturb the cultures for at least 16 hours*
13. Microscopically examine the cultures and pre-equilibrate Human Large Vessel Endothelial Cell Growth Medium
14. After 16-24 hours, aspirate the culture medium and replace with fresh, pre-equilibrated medium (5ml per 25cm^2), dispensing carefully over a cell-free surface to avoid any risk of damaging or dislodging the cells
15. Return the flask(s) to a 37°C, 5% CO_2 humidified incubator and refresh culture medium every 48-72 hours until cells reach 60-90% confluency
    N.B. *Ideally passage the cells when they are still actively dividing*

Caution:

- **All human cells should be treated as potentially infectious. Wear appropriate personal protective equipment. Use appropriate disposal methods for potentially pathogenic or biohazardous material.**
- **For research use only. Not for diagnostic or therapeutic use.**