ANGIOCRINE BIOSCIENCE

Product Instruction Sheet

Please read first

VeraVec™ HUAEC (HVERA105)

INTENDED USE

These *VeraVec*™ Human Endothelial Cells are intended for research use only. It is not intended for any diagnostic, therapeutic or other application in either humans or animals.

QUALITY CONTROL REPORTS

Each Lot is quality controlled for viability, purity, and pathogens. Please visit www.angiocrinebioscience.com with your lot number and order number to view this information.

CONTACT INFORMATION

Please contact support@angiocrinebio.com with questions on the handling of *VeraVec* cells.

CITATION OF *VeraVec* HUMAN CELLS

When publishing, please cite as Angiocrine Bioscience *VeraVec* Human Endothelial Cells.

ANGIOCRINE BIOSCIENCE WARRANTY

The viability of Angiocrine Bioscience products is warranted for 30 days from the date of shipment and is only valid if the storage and culturing instructions listed here are strictly followed. Deviations from these protocols may result in undesired results and are not covered by this warranty.

DISCLAIMER

All products distributed by Angiocrine Bioscience are covered by a Limited Use License. For a description of these terms and the most up-to-date information regarding Angiocrine Bioscience products, visit www.angiocrinebioscience.com.

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DESCRIPTION

Organism: Homo Sapiens
Tissue: Umbilical Cord
Cell Type: Arterial Endothelial Cell
Disease: Normal
Quantity: ≥ 1 million cells
Growth Conditions: Adherent
Materials Needed:

- Sterile, cell culture treated plastic dishes or plates, area=25cm^2
- Class II (or higher) cell culture hood
- Standard personal protective equipment (PPE: gloves, lab coat, safety eyewear)
- Sterile alcohol wipes
- Centrifuge
- Media and cytokines (see Media Formulations below for recipes)
- Fibronectin
- Phosphate buffered saline (PBS)
- Accutase (or a similar tissue-culture dissociation solution)
- 15ml conical tubes
- Sterile serological pipets
- 37˚C water bath
- Cell culture incubator
- Sharps collection container
- Biohazard waste collection

PROCEDURES

Receipt and Storage

- Caution: Cells are packaged in dry ice. Handle with PPE.
- Inspect the packaging and vial for damage or thawing.
- If the cells are to be cultured immediately, proceed to the instructions on thawing cells.
- If the cells are to be stored, place in cryogenic storage at a temperature lower than -130˚C. Liquid nitrogen storage is preferable. Vials are liquid-nitrogen safe.

Thawing Cells

- Adorn proper PPE.
- Warm the appropriate media to 37˚C.
- Pre-treat tissue culture flask with 1µg/ml fibronectin made in PBS. After 20 minutes aspirate the fibronectin/PBS solution from the flask.
- Place ampule of cells in water bath until thawed. This should take less than 2 minutes - do not leave the cells in the water bath for longer than necessary.
- Sterilize the ampule with an alcohol wipe.
- Place in a clean and uncluttered cell culture hood.
  - Wrap the vial in the alcohol wipe and grasp firmly.
  - Push the top stem of the ampule forward with thumb slowly but firmly until the ampule breaks.
  - Carefully remove contents with a pipette and place in a 15ml conical tube.
  - Dispose of glass ampule in biohazardous sharps waste container.
- Add 9mls of media and invert gently several times.
- Spin at 400G for 5 minutes at 4˚C.
- Carefully aspirate supernatant without disturbing the pellet.
- Resuspend the pellet by pipetting gently several times with 6 mls of media.
- Transfer the cell suspension into a T25 cell culture treated flask, or dish of similar size.
- Place the cells in humidified 37˚C cell culture incubator with 5% CO_2 [5% O_2 recommended, but not required].
- Refeed the cells the following day with fresh media.

Splitting Cells

- Allow the mouse *VeraVec* cells to approach 100% confluence.
- NOTE: Mouse *VeraVec* cells are growth inhibited by contact and can remain at 100% confluence for extended periods without overgrowth or detriment. However, it is suggested to replace the media every 3-4 days.
- Warm the appropriate media to 37˚C.
- Warm cell culture grade dissociation enzyme to 37˚C. Such enzymes include Accutase, Trypsin or Collagenase.

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PROCEDURES *(continued)*

Splitting Cells *(continued)*

- Rinse the cells one time with PBS.
- Add the minimal amount of enzyme solution to cover the cells.
- Return to the incubator and check every 2-3 minutes.
- When the cells have detached from the surface, transfer cell suspension into a 15ml conical tube.
- Add an appropriate volume of media to the flask/dish (at least 2X the volume of dissociation enzyme solution.) Spin at 400G for 5 minutes at 4˚C.
- Carefully aspirate supernatant without disturbing the pellet.
- Resuspend by pipetting gently several times with media.
- Transfer the cell suspension into a new tissue cultured treated plate(s) or flask(s).*Place the cells in humidified 37˚C cell culture incubator with 5% CO_2 [5% O_2 recommended, but not required].

**NOTE: The format for passaging cells is at the discretion of the user. As a general guide, it is suggested that mouse VeraVec cells are split at a ratio of no greater than 3 to 1. For example, 1 confluent T75 flask of mouse VeraVec cells can be split into 3 new T75 flasks. These new T75 flasks should reach confluency within 4-6 days.*

MEDIA FORMULATIONS

All media should be prepared under sterile conditions, filtered through a .22 micron filter, and stored at 4˚C in the dark. Although not required we recommend the use of Normocin (InvivoGen catalog # ant-nr-1) added to all medias listed below. Prepared media has a shelf-life of at most four weeks. We suggest preparing 500ml bottles of media as follows:

Human Complete EC media for human *VeraVec* cells:

- Medium 199 (Hyclone: #SH30253.01)
- Endothelial cell supplement (Biomedical Technologies: #BT-203) (final concentration: 50µg/ml)
- Fetal Bovine Serum (Omega Scientific: #FB-11) (final concentration: 20%)
- Antibiotic-Antimycotic 100X-solution (Invitrogen: #15240-062)
- HEPES buffer (Invitrogen: #15630-080) (final concentration: 10mM)
- Heparin (Sigma: #H3149-100KU) (final concentration: 50µg/ml)
- Glutamax 100X-solution (Life Technologies: #35050061)

Notes: Human Complete EC media can be used for initial thawing and plating of human VeraVec cells and HUVECs, as well as normal maintenance and outgrowth of human VeraVec cells and HUVECs. Media should be changed every 2 days.

Human KOSR ('serum-free') EC media for human *VeraVec* cells:

- Medium 199 (Hyclone: #SH30253.01)
- Knock-out Serum Replacement (Life Technologies: #10828028) (final concentration: 20%)
- Antibiotic-Antimycotic 100X-solution (Invitrogen: #15240-062)
- HEPES buffer (Invitrogen: #15630-080) (final concentration: 10mM)
- SB431542 small molecule (R&D Cat # 1614) (final concentration: 5µM – see below for preparation)
- Heparin (Sigma: #H3149-100KU) (final concentration: 50µg/ml)
- Glutamax 100X-solution (Life Technologies: #35050061)
- *FGF-2 (Peprotech: #100-18B) (final concentration: 20ng/ml – add fresh when changing media)
- *VEGF (Peprotech: #100-20) (final concentration: 10ng/ml – add fresh when changing media)
- *Preparation of SB431542: Resuspend 10mg in 470µl of DMSO. Alliquot the solution into 50µl units and store at -20ºC (keep exposure to light at a minimum). Add one unit (50µl) to a 500ml bottle of media for *VeraVec* ECs as described above for a final concentration of 5µM.

**Notes: FGF-2 and VEGF should be kept at 4˚C for no more than 1 week. Therefore, we recommend that the user DOES NOT mix them in with the 500ml Human KOSR ('serum-free') EC media bottle, but rather add these cytokines directly to the cells whenever the media is changed, which should be done every 2 days. This is a serum-free media that is ideal if the user desires well-defined culture conditions. It can be used to maintain and grow out human VeraVec HUVECs already in culture, however it is recommended that Human Complete EC media be used if serum-free conditions are not necessary. Additionally, Human KOSR ('serum-free') EC media is NOT ideal for Naïve HUVECs.*

Human X-Vivo ('serum-free') EC media for human *VeraVec* cells:

- X-Vivo media (Lonza: #04-448Q)
- Antibiotic-Antimycotic 100X-solution (Invitrogen: #15240-062)

Notes: Human X-vivo ('serum-free') EC media can be used to maintain human VeraVec cells AFTER they have been plated into desired tissue-culture format for terminal experimental use. This media should NOT be used for initial thawing and plating of VeraVec cells. This is a serum-free, cytokine-free, heparin-free media that is capable of sustaining VeraVec cells in the most basic culture conditions. Cells can be maintained in Human X-vivo EC media for beyond 1 week, but instead media should be changed every 2 days. Cells should NOT be passaged in Human X-vivo EC media, nor should the user expect outgrowth of human VeraVec cells when using this media. However, human VeraVec cells cultured in Human X-vivo EC media can be re-cultured in either Human Complete EC media or Human KOSR EC media, at which point they can once again be passaged and grown out.