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EndoGRO™ Human Umbilical Vein Endothelial Cells (HUVEC)

CATALOG NUMBER: SCCE001

LOT NUMBER:

QUANTITY: 1 ampoule containing 500,000 cells

BACKGROUND: HUVEC are a commonly studied human cell type extracted from human neonatal umbilical cords. They are relatively easy to culture, and provide a valuable cell model for many vascular biology research applications, including inflammation, angiogenesis, atherosclerosis, blood clotting, vasoconstriction, and vasodilation. EndoGRO™ HUVEC are provided at passage 1, and have been quality tested in low-serum media without exposure to phenol red.

This product is for Research Use Only. This product is not approved for human or veterinary use, or for use in *in vitro* diagnostic or clinical procedures.

STORAGE: EndoGRO HUVEC are sold as cryopreserved ampoules. Ampoules are shipped in insulated packages containing dry ice to insure the cells remain in a cryopreserved state. To maintain the cells' integrity, unpack the products immediately upon receipt. The cryopreserved cells should be placed in the liquid or gas phase of a liquid nitrogen dewar for storage. If the cells are to be thawed and plated within 24 hours they may be stored at -80°C.

Do not store the ampoule for more than 24 hours at -80°C as the cells will slowly degrade at this temperature. Long-term storage of the ampoule must be in a liquid nitrogen dewar or a mechanical freezer designed for cryopreserved cell storage that maintains a temperature below -135°C.

Millipore recommends storing the cryopreserved vials in liquid nitrogen vapor phase. Handle cryopreserved vials with caution. Always wear eye protection and gloves when working with cell cultures. Aseptically vent any nitrogen from cryopreserved vials in a biosafety cabinet prior to thawing the vials in a water bath. If vials must be stored in liquid phase, the vials should be transferred to vapor phase storage or -80°C for at least 24 hours prior to being thawed.

CELL CULTURE PROTOCOL OVERVIEW:

1) Obtain cryopreserved HUVEC from storage and thaw. Place thawed proliferating HUVEC in incubator at 37°C 5% CO_2.
2) Feed HUVEC using pre-warmed EndoGRO reduced-serum medium according to feeding chart.
3) When HUVEC are 80-100% confluent and actively proliferating, passage cells.
4) Rinse cultures with buffered saline solution and trypsinize cells until rounded, do not over trypsinize.
5) Add a trypsin neutralizing solution to stop trypsinization.
6) Centrifuge at 150 x g for 3-5 minutes. Adjust speed and time as appropriate for your centrifuge.
7) Aspirate solution from centrifuge tube; add pre-warmed EndoGRO medium, and gently re-suspend HUVEC.

8) Count HUVEC using a hemocytometer, re-plate at 2,500-5,000 per cm^2 in vessel containing pre-warmed EndoGRO medium.

9) Incubate HUVEC using 1 mL of EndoGRO Medium per 5 cm^2 at 37°C 5% CO_2.

10) Always use a certified biological safety cabinet when working with cells or media.

THAWING AND PLATING CRYOPRESERVED CELLS:

Remove ampoule from dewar and check cap to be sure ampoule is securely sealed. Hold ampoule in a 37°C water bath so that only the bottom half of the ampoule is in the water. To avoid potential contamination, do not allow the ampoule cap to make contact with the water. This procedure should take approximately one minute or until ampoule is slightly thawed, do not over thaw, as this may damage cells. Place ampoule in a biological safety cabinet and sterilize the exterior of the ampoule using 70% EtOH. Carefully remove the cap to avoid contamination or spatter. Gently re-suspend the cells in the ampoule using a 1 or 2 mL sterile pipette. Do not centrifuge; the cells may be directly plated from the ampoule. Plate cells into pre-warmed EndoGRO Medium in desired culture vessel at a density of 2,500 to 5,000 cells per cm^2. Gently swirl the culture vessel to evenly distribute cells within the vessel. Place seeded culture vessel in the incubator at 37°C 5% CO_2.

MEDIA PREPARATION: EndoGRO HUVEC should be cultured in EndoGRO medium (warmed to 37°C) for best results. Please see specific media data sheets for further information (SCME001).

PASSAGING CELLS: Normal HUVEC may be passaged when the culture is 80-100% confluent and actively proliferating. HUVEC are not contact inhibited; however, it is recommended that endothelial cells be passaged before reaching confluence as post-confluent HUVEC may exhibit slower proliferation after passaging.

1. Aspirate the medium from the culture vessel. Rinse the flask with a buffered saline solution by adding 1 to 2 mL per cm^2 and gently tilting the flask to cover entire surface. Aspirate the buffered saline solution from the culture vessel, repeat the rinse if desired.
2. Add 0.5 to 2 mL of a 0.05% Trypsin/0.02% EDTA solution to the vessel for each 25 cm^2. If using more than 1.0 mL per 25 cm^2, aspirate the Trypsin/EDTA solution to leave a thin film covering the cells; do not aspirate to dryness. Observe the HUVEC carefully under the microscope. When the HUVEC round up, they are ready to be detached. This normally takes from 1-3 minutes depending on the confluence level. Do not over trypsinize as this will damage the cells.
3. Detach the HUVEC by gently tapping the culture vessel from several sides. Once the HUVEC appear to be detached, add a trypsin neutralizing solution using a volume equal to the amount of Trypsin/EDTA that was used. Gently swirl to ensure all of the trypsin solution is neutralized.
4. Using safe laboratory techniques, pipette the HUVEC into a sterile centrifuge tube. Add a buffered saline solution to the culture vessel and collect additional HUVEC. Add the second collection of HUVEC to the sterile centrifuge tube with the other cells.
5. Check culture vessel under the microscope for cells still attached and repeat steps if necessary to retrieve all the cells from the vessel.
6. Centrifuge HUVEC in a refrigerated centrifuge at 150 X g for five minutes. For best results, calculate speed and time for individual centrifuge type. Do not over centrifuge cells as this will damage them.

7. After centrifugation, the cells should form a clean loose pellet. Aspirate neutralized trypsin from the centrifuge tube and resuspend the cell pellet in pre-warmed EndoGRO medium by gently pipetting up and down with a 2 or 5 mL pipette.

All steps must be completed under sterile conditions in a biological safety cabinet.

STANDARD CALCULATION FOR PASSAGING CELLS:

1. After passaging of the cells, resuspend the centrifuged cell pellet in 2-8 mL of pre-warmed EndoGRO™ Medium. Gently resuspend the HUVEC evenly in the media.
2. Using sterile technique and a clean hemocytometer, remove 10 µL of the HUVEC suspension and place on the hemocytometer. Count a minimum of four quadrants on the hemocytometer. For accurate cell counts, the optimal number of HUVEC per quadrant should be between 25-50 cells.
3. Make a dilution of the cells if necessary to achieve a more accurate cell count. After counting the HUVEC, average the four quadrants. Take the HUVEC count average and multiply by the 2 mL used for the original dilution of the centrifuged cells and multiply by 10^4 to get the total number of cells per mL in the cell suspension. Divide the total number of HUVEC by the recommended seeding density of 2,500-5,000 HUVEC per cm^2. This will provide you the total area you are able to plate with the cells.
4. Inoculate the HUVEC into the culture vessels prepared with pre-warmed EndoGRO Medium. Swirl gently to evenly distribute the HUVEC and place culture vessels into the incubator at 37°C 5% CO_2.

RECOMMENDED FEEDING GUIDELINES:

The following guidelines are for a T-25 flask. Adjust volumes according to culture surface area.

Cultures under 20% confluent (to be re-fed in 2 days)	Re-feed with 5 mL of warmed medium
Cultures under 20% confluent (to be re-fed in 3 days)	Re-feed with 7 mL of warmed medium
Cultures 20-30% confluent (to be re-fed or passaged in 2 days)	Re-feed with 8 mL of warmed medium
Cultures over 30% confluent (to be passaged in 2 days)	Re-feed with 7-10 mL of warmed medium

RELATED PRODUCTS:

Product	*Catalog Code*
EndoGRO-LS Complete Medium	SCME001
EndoGRO-VEGF Complete Medium	SCME002
EndoGRO-MV-VEGF Complete Medium	SCME003
EndoGRO-MV Complete Medium	SCME004

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