Endothelial Cell Growth Media without VEGF

Media for the Low-serum, VEGF-free Expansion of Primary Endothelial Cells

Catalog Number: CCM029

Volume: 250 mL

PRODUCT DESCRIPTION

Primary endothelial cells are commonly used for the study of developmental and disease mechanisms involving blood vessels, such as angiogenesis. Endothelial cells are phenotypically characterized as $CD31^+$, KDR^+, and vWF^+ cells.

INTENDED USE

Endothelial Cell Growth Media without VEGF is formulated to support the expansion of human primary endothelial cells under low-serum, VEGF-free conditions. The media has been tested for its ability to support $CD31^+$, KDR^+, and vWF^+ human umbilical vein endothelial cell growth *in vitro*.

MATERIALS PROVIDED & STORAGE

Note: ***The components for this kit require different storage/shipping temperatures and will arrive in separate packaging.***

PART	PART #	DESCRIPTION	STORAGE OF UNOPENED MATERIAL	STORAGE OF OPENED/ DILUTED MATERIAL
Endothelial Cell Growth Base Media	390598	250 mL of 1X media.	Store under sterile conditions at 2-8 °C.*	Store at 2-8 °C.*
Endothelial Cell Growth Supplement without VEGF (50X)	390634	5 mL of a 50X concentrated solution containing optimized concentrations of: - Human FGF basic - Human LR3 IGF-1 - Human EGF - Heparin - Hydrocortisone - L-Ascorbic Acid - Fetal Bovine Serum	Store under sterile conditions at ≤ -20 °C in a manual defrost freezer.*	Store at 2-8 °C for up to 1 month, or aliquot and store ≤ -20 °C in a manual defrost freezer.*

* Provided this is within the expiration date of the media.

PRECAUTION

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- These reagents should not be used beyond the expiration date indicated on the label.
- Results may vary due to variations among primary endothelial cells originating from different donors.

PROCEDURE FOR THE EXPANSION OF HUMAN PRIMARY ENDOTHELIAL CELLS

The protocol below describes the expansion of human umbilical vein endothelial cells (HUVECs) in Endothelial Cell Growth Media without VEGF.

Note: *This protocol must be read in its entirety before using this product.*

OTHER MATERIALS REQUIRED

- HUVECs
- Penicillin-Streptomycin (100X), optional
- TrypLE™ Express
- Sterile Phosphate-Buffered Saline (PBS) (Tocris®; Catalog # 3156)
- 75 cm^2 tissue culture (T75) flasks
- 15 mL centrifuge tubes
- Serological pipettes
- Pipette and pipette tips
- 37 °C and 5% CO_2 humidified incubator
- Centrifuge (low speed clinical or equivalent)
- Hemocytometer
- Inverted Microscope
- Water bath

REAGENT PREPARATION

Endothelial Cell Growth Supplement without VEGF- Thaw the Endothelial Cell Growth Supplement without VEGF (50X) at 2-8 °C or room temperature.

Complete Endothelial Cell Growth Media without VEGF – Add 5 mL of Endothelial Cell Growth Supplement without VEGF to 250 mL of Endothelial Cell Growth Base Media. Add Penicillin-Streptomycin at a 1:100 dilution. Store under sterile conditions at 2-8 °C for up to 2 weeks. **Note:** *If Penicillin-Streptomycin is not needed for the experiment, it can be omitted.*

PROCEDURE

Culturing of HUVECs

1. Warm Complete Endothelial Cell Growth Media without VEGF to 37 °C.
2. Determine the size and number of flasks needed for plating. Cells should be plated at 6.7 x $10^3/cm^2$. For example, 0.5 x 10^6 cells/T75 flask.
3. Warm the frozen vial of HUVECs until just thawed and then, immediately and gently, transfer the cells to a 15 mL centrifuge tube containing at least 5.0 mL of pre-warmed Complete Endothelial Cell Growth Media without VEGF.
4. Centrifuge at 200 x g for 5 minutes.
5. Remove the supernatant and resuspend the pellet in an appropriate amount of pre-warmed Complete Endothelial Cell Growth Media without VEGF.
6. Add the HUVEC suspension to the flasks in a total of 15 mL/T75 flask.
7. Change media the day following thaw.
8. Monitor cells daily and change media every other day. Passage the cells at 80-90% confluency.

Subculturing of HUVECs

1. Warm Complete Endothelial Cell Growth Media without VEGF to 37 °C.
2. Remove and discard the media from the flasks. Rinse plate once with sterile 1X PBS.
   Note: *Do not dispense the PBS directly onto the cells during washing so as not to disrupt the cells.*
3. Add enough TrypLE™ Express to just cover the cells (2.0 mL/T75 flask). Gently rock the flask to disperse the solution evenly over the cells.
4. Incubate the flask at 37 °C for 1-2 minutes. Monitor periodically for cell detachment by observing the cells under the microscope. Tap the side of the flask to aid the detachment of the cells.
5. Transfer the cell suspension to a 15 mL centrifuge tube containing 8 mL of pre-warmed Complete Endothelial Cell Growth Media without VEGF and centrifuge at 200 x g for 5 minutes.
6. Plate cells (approximately 6.7 x 10^3 cells/cm^2) on desired number of flasks.
7. Change media the day following split.
8. Monitor cells daily and change media every other day. Passage the cells at 80-90% confluency.

DATA EXAMPLES

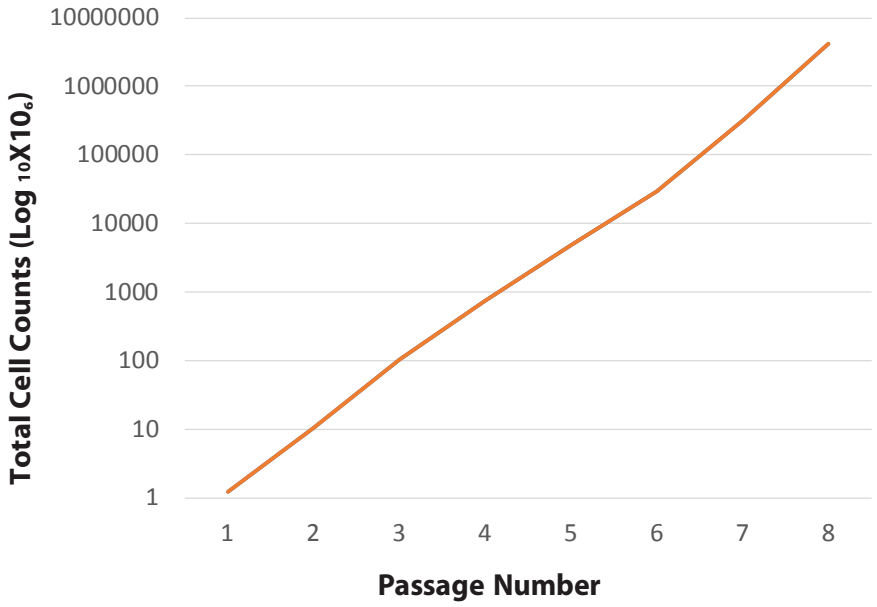

Figure 1: Endothelial Cell Growth Media without VEGF Supports Cell Proliferation of Human Umbilical Vein Endothelial Cells (HUVECs). One well of a 6-well plate of HUVECs (0.13 x 10^6 cells) was expanded over 8 passages using Complete Endothelial Cell Growth Media without VEGF. Cells were passaged every 4-6 days. The histogram shows the cumulative number of HUVECs expanded over 8 passages if all cells were replated at each passage.

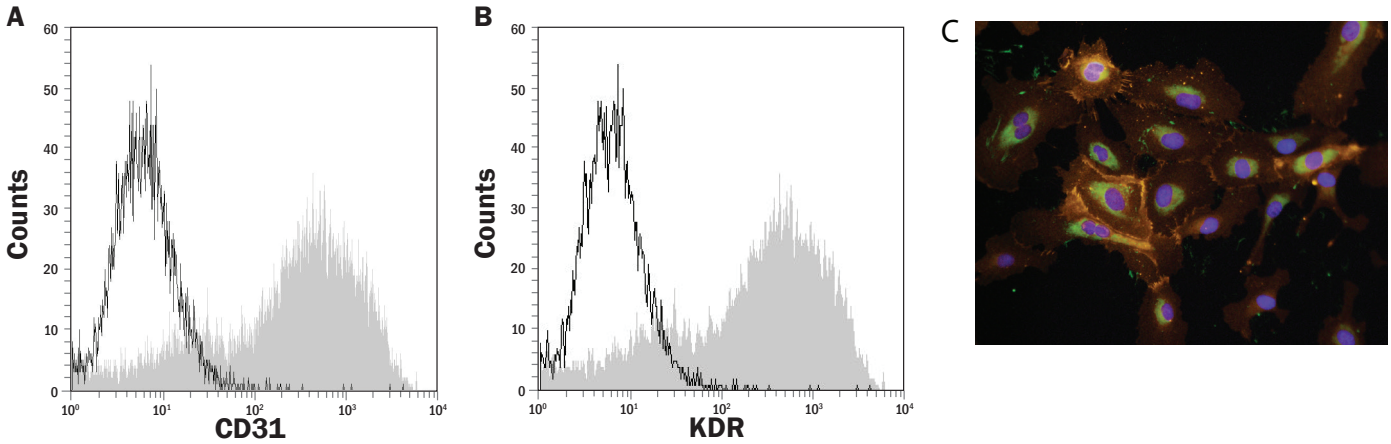

Figure 2: Human Umbilical Vein Endothelial Cells (HUVECs) Express Endothelial Cell Markers CD31, KDR, and vWF. HUVECs were expanded in Complete Endothelial Cell Growth Media without VEGF and stained for marker expression. **(A,B)** Flow cytometry analysis of endothelial cell marker expression. Filled histograms indicate cells stained with Mouse Anti-Human CD31 PE-conjugated Antibody (A; R&D Systems®, Catalog # FAB3567P) or Mouse Anti-Human KDR PE-conjugated Antibody (B; R&D Systems®, Catalog # FAB357P). Open histograms indicate staining with PE-conjugated Mouse IgG_1 isotype control (R&D Systems®, Catalog # IC002P). **(C)** Immunocytochemistry analysis of endothelial cell marker expression. Cells were double stained with Mouse Anti-Human CD31 Monoclonal Antibody (red; R&D Systems®, Catalog # BBA7) and Rabbit Anti-Von Willebrand Factor (vWF) (green; Novus Biologicals®, Catalog # NB600-586). The nuclei were counterstained with DAPI (blue).

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