ESSEN BioScience

PRODUCT DATA SHEET

CellPlayer™ Angiogenesis HUVEC CytoLight Green

Essen BioScience Catalog Number: 4453

Storage

Liquid Nitrogen

Note: Cells can be thawed and cultured immediately upon receipt or stored in liquid nitrogen for long-term storage. Storage at -80°C is not recommended.

Presentation

1mL, 1.7 x 10^5 cells/mL in 90% FBS, 10% DMSO

Background

Each vial contains 170,000 Normal Human Umbilical Vein Endothelial Cells (HUVEC), pooled from multiple donors. Prior to cryopreservation, the parental HUVEC are lentivirally infected to stably express CytoLight Green (Lenti, CMV) driven off of a CMV promoter. This results in a **>90%** transduction efficiency of the HUVEC population as measured by flow cytometry analysis. Upon thawing, the HUVEC CytoLight Green are guaranteed to be ≥ 70% viable. Normal human cells have a limited life span *in vitro*. When cultured according to protocol, these HUVEC CytoLight Green can achieve several population doublings.

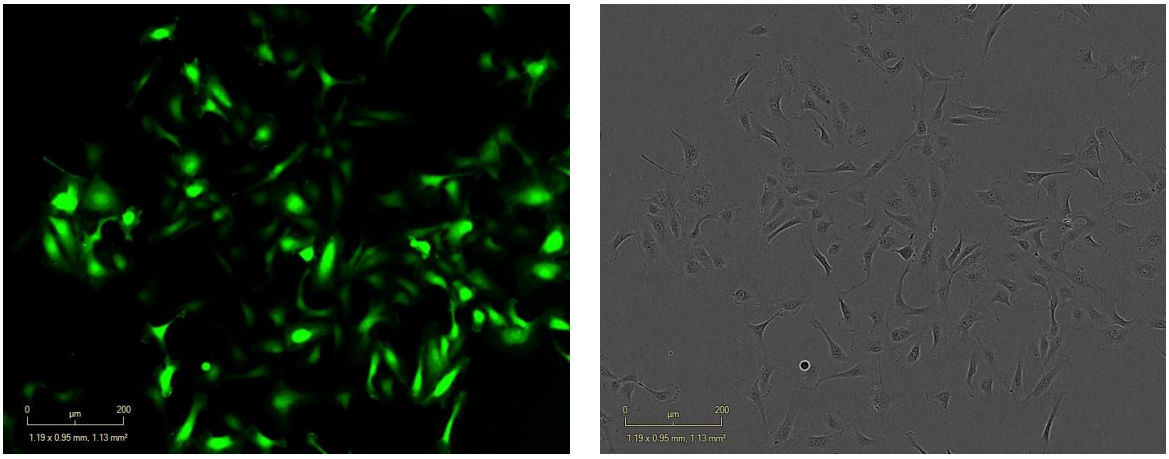

Figure 1. HUVEC CytoLight Green Line. Left: CytoLight Green Expression; Right: Phase Contrast. Scale bar: 200µm.

Thawing and Culturing Cells

1. The recommended seeding density for HUVEC CytoLight Green is 2,500 to 5,000 cells/cm^2. For example, one vial of HUVEC CytoLight Green is sufficient to seed 1 to 3 T25 flasks. From this, calculate the number of flasks needed.

2. Prepare the flasks and culture media. To each 500mL bottle of EBM-2, add the desired EGM-2 bullet kit supplements to make EGM-2 complete media. Add 1 mL of media per 5 cm^2 of flask area. For example, add 5 mL media per T25 flask. Allow the flasks to equilibrate for at least 15 minutes in a 37°C, 5% CO_2 humidified tissue culture incubator.

3. Remove the vial containing HUVEC CytoLight Green from liquid nitrogen.

4. In a 37°C water bath, quickly thaw the vial by gentle agitation. Be careful not to submerge entire vial to avoid contamination. This process should take no more than 2 minutes. Remove vial when only a tiny ice crystal remains.

5. Wipe vial with 70% ethanol. In a biosafety hood, aliquot cells into sufficient EGM-2 complete media to distribute among the flasks set up in Step 2. For 3 flasks, add 1 mL cell suspension to 2 mL EGM-2 complete media and distribute 1 mL into each flask.

6. Gently rock the culture flasks to evenly distribute the cells and place in 37°C incubator.

Maintaining Cell Culture

1. Completely change the growth media the day after seeding and every other day thereafter.

2. Warm the appropriate amount of media in 37°C water bath. Remove the old growth media and replace with fresh media and return to incubator.

Subculturing Cells

1. HUVEC CytoLight Green should be subcultured when they are 70-80% confluent.

2. In a 37°C water bath, warm the amount of Trypsin/EDTA, PBS, and EGM-2 complete media required for successful passage of each T25 culture flask.

3. Aspirate the old media. Gently wash the flask with 3 to 5 mL room temperature PBS. This is important to remove any remaining proteins in the media that neutralize trypsin. After the wash, aspirate the PBS.

4. Add 2 mL Trypsin/EDTA solution to each T25 flask. Place the flask flat on the culture hood until 90% of the cells have lifted. This should take 2-5 minutes. Release the majority of cells by hitting the flask against the palm of your hand.

5. After the cells have detached, add 4 mL of room temperature EGM-2 to neutralize the trypsin. Wash the flask several times with the media/trypsin solution to harvest as many cells as possible.

6. Transfer the detached cells to a 15 mL conical tube and centrifuge the harvested cells at 220 x g for 5 minutes to pellet the cells.

7. Resuspend the cell pellet in 4 to 5 mL of EGM-2 complete growth media and count the cells using a hemacytometer.

8. Plate the cells into the new vessel of choice at the appropriate density in fresh EGM-2 complete growth media.

Freezing Cells

1. Thaw and warm FBS in a 37°C water bath prior to harvesting the cells. Also, equilibrate tissue grade DMSO to room temperature.

2. Lift cells as described in Steps 1-6 of "Subculturing Cells" section with the following exception. Prior to placing in the centrifuge remove a 20-100µL aliquot to count on the hemacytometer.

3. Count the cells using the hemacytometer to calculate the total number of cells to be frozen.

4. Determine the cell freezing density per vial. It is recommended to freeze cells between 1 x 10^5 and 1 x 10^6 cells/mL.

5. Divide the number of total cells by the desired cell density to determine the number of vials to be frozen and amount of freezing media required.

6. Make the appropriate amount of freezing media (90% FBS/10% DMSO) for the number of vials. It is good practice to make a few mL extra in case of volume loss during the procedure. For 10 mL freezing media, mix 9 mL FBS with 1 mL DMSO.

7. After centrifugation, aspirate media and resuspend in volume of freezing media calculated in Step 5.

8. Triturate cells several times to ensure a homogenous cell mixture.

9. Aliquot 1 mL per vial into a 2 mL cryovial.

10. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).

11. Transfer frozen cells to liquid or vapor-phase nitrogen for long-term storage. Temperatures should be between -125°C and -200°C.

Related Products

Cat.# 4452 CellPlayer Angiogenesis PrimeKit – Cryo
Cat.# 4436 CellPlayer Angiogenesis PrimeKit – Live
Cat.# 4437 CellPlayer VEGF/Suramin Supplement Kit
Cat.# 4438 CellPlayer bFGF/Suramin Supplement Kit
Cat.# 4439 CellPlayer EGF/Suramin Supplement Kit
Cat.# 4541 CellPlayer Angiogenesis Optimized Media Kit
Cat # 4410 CellPlayer Angiogenesis Assay Media 500 mL

For additional information on this and other products, please contact Essen BioScience at: sales@essenbio.com.

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