NEUROMICS

Cas9-Human Retinal Microvascular Endothelial Cells

Catalog #: HEC09-CAS

Cell #: >5x10^5 cells

Storage: Liquid Nitrogen until ready for culture.
While Culturing keep in 37°C CO_2 incubator

Product Format: Frozen Vial

GENERAL INFORMATION

Cas9 is an enzyme commonly used in the CRISPR-Cas9 gene editing system to cut DNA at specific locations. Stable expression of Cas9 is desirable in gene editing experiments, as it allows for precise and consistent genome editing.

Human retinal microvascular endothelial cells (HRMECs) have been genetically modified to express Cas9 protein in a stable manner. This means that the cells have been engineered to continuously produce and maintain the expression of the Cas9 protein over time. These cells were isolated from the normal human retinal tissue before Cas9 protein was introduced into the cell. The stable expression enables researchers to make targeted changes to the genome of the cell.

The cells are shipped in frozen vials (the cells are provided @ passage 1).

Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

HANDLING OF ARRIVING CELLS

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells into a -80°C freezer for short-term storage or liquid nitrogen tank for long-term storage.

3. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

PRODUCT TESTING

- Negative for bacteria, yeast, fungi, and mycoplasma
- Cytoplasmic VWF/Factor VIII >95% positive by immunofluorescence
- Cytoplasmic uptake of Di-I-Ac-LDL >95% positive by immunofluorescence
- Cytoplasmic PECAM1 >95% positive by immunofluorescence

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MEDIUM

We recommend Cas9 Endothelial Growth Medium (cat. MED004) for these cells. The growth medium contains ingredients to support Cas9 activity.

PROTOCOL FOR THAWING THE CELLS

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

1. Add 2 mL of AlphaBioCoat (cat. AC001) to a T25 flask to cover the whole surface of the flask. 5 minutes later, dispose of the excess coating solution by aspiration, and rinse the plate with 1xPBS. Discard the 1xPBS, and the flask is ready to be used.

2. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

3. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

4. Remove the vial from the water bath as soon as the contents are thawed. Decontaminate by dipping in or spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.

5. Transfer the cells into the pre-coated T25 flask with 10 mL of growth media.

6. Incubate the culture at 37°C in a suitable incubator. A 5% CO2 in air atmosphere is recommended if using the medium described on this product sheet.

SUBCULTURING PROCEDURE

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

1. To passage the cells, rinse the cells in a T25 flask with 5 mL HBSS (RT) twice; then add 2 mL Cell Detachment Solution (cat. ADF001) into one T25 flask. Gently dispose of the excessive Cell Detachment Solution within 20 seconds by aspiration.
2. Leave the T25 flask with the cells at RT or 37C for 1 min (most cells usually will detach from the surface within 1-2 mins) or monitor the cells under a microscope until most of the cells become rounded up, and then gently tap the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under a microscope.
3. Add 5ml Trypsin Neutralization solution and spin down the cells with 800g centrifugation for 5 mins.
4. Resuspend the cell pellet with 10 or 15 ml medium and transfer 5 ml each into 2 or 3 pre-coated T25 flasks (for 1/2 to 1/3 subculture ratio).
5. Change the medium every 2 or 3 days, and the cells usually become confluent within 7 days (when split at a 1/3 ratio).
6. To prepare quiescent cells, when cells are nearly confluent, replace growth media with Cas9-Basal Medium (cat. MED005) for about 8-12 hours before your experiments.

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Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended

Medium Renewal: Every 2 to 3 days

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