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Endothelial Nitric Oxide Synthase Knockout (eNOS KO) Mouse Primary Pulmonary Vein Endothelial Cells

Catalog No. **eNOS-6060**

Suggested Medium: Endothelial Cell Medium /w Kit – 500 ml
Catalog No. **M1168**

Product Description

eNOS KO Mouse Primary Pulmonary Vein Endothelial Cells from *Cell Biologics* are isolated from pulmonary vein tissue of pathogen-free laboratory mice. eNOS KO Mouse Primary Pulmonary Vein Endothelial Cells are grown in T25 tissue culture flasks pre-coated with gelatin-based coating solution for 2 min and incubated in *Cell Biologics'* Culture Complete Growth Medium generally for 3-7 days. Cultures are then expanded. Prior to shipping, cells are detached from flasks and immediately cryo-preserved in vials. Each vial contains at least $1x10^6$ cells per ml and are delivered frozen. The method we use to isolate primary endothelial cells was developed based on a combination of established and our proprietary methods. These cells are pre-coated with PECAM-1 antibody, following the application of magnetic pre-coated with secondary antibody.

Product Testing

eNOS KO Mouse Primary Pulmonary Vein Endothelial Cells are tested for expression of markers using antibody, VE-cadherin (CD144, VE-cadherin Antibody, C-19, sc6458, Santa Cruz); AF1002 (R&D System) or CD31/PECAM-1 (Purified Rat Anti-Mouse CD31, Catalog No. 553370, BD) by immunofluorescence staining or FACS. eNOS KO Mouse Primary Pulmonary Vein Endothelial Cells are negative for bacteria, yeast, fungi and mycoplasma. Cells can be expanded for 3-6 passages at a split ratio of 1:2 under the cell culture conditions specified by *Cell Biologics.* Repeated freezing and thawing of cells is not recommended.

Laboratory Applications

eNOS KO Mouse Primary Pulmonary Vein Endothelial Cells can be used in assays of cell-cell adhesion, migration, vascular tube formation, or transendothelial resistance (TER). Standard biochemical procedures performed with endothelial cell cultures include RT-PCR, Western blotting, immunoprecipitation, immunofluorescent staining, immunofluorescent flow cytometry, or generating cell derivatives for desired research applications.

Storage of *Cell Biologics'* Products

Cell Biologics ships frozen cells on dry ice. On receipt, immediately transfer frozen cells to liquid nitrogen (-180 °C) until ready for experimental use. Live cell shipment is also available on request.
Never can primary cells be kept at -20 °C.

Authorized Uses of *Cell Biologics'* Products

eNOS KO Mouse Primary Pulmonary Vein Endothelial Cells from *Cell Biologics* are distributed for research purposes only. Our products are not authorized for human use, for in vitro diagnostic procedures or for therapeutic procedures. Transfer or resale of any *Cell Biologics'* cells or products from the purchaser to other markets, organizations or individuals is prohibited by *Cell Biologics* without the company's written consent.
Cell Biologics' Terms and Conditions must be accepted before submitting an order.

Disclaimer

Although eNOS KO Mouse Primary Pulmonary Vein Endothelial Cells are isolated from laboratory mice testing pathogen-free, investigators should handle the cells that they receive from *Cell Biologics* with caution and treat all animal cells as potential pathogens, since no test procedure can completely guarantee the absence of infectious agents.

Warranty and Liability

Cell Biologics' guarantee applies only to your purchase of *Cell Biologics'* cells with *Cell Biologics'* Media and Coating Solution for appropriate cell culture and cell testing following *Cell Biologics'* online protocols within 35 days from the date of product delivery.

Primary Cell Culture Protocol

All cell culture procedures must be conducted in a bio-safety cabinet.
Any and all media, supplements, and reagents must be sterilized by filtration through a 0.2 µm filter.
Use aseptic technique to prevent microbial contamination.
Cryo-preserved cells must be stored in liquid nitrogen or seeded immediately upon arrival.

Medium:
Review the information provided on the *Cell Biologics* website about appropriate culture media (e.g. serum and other supplements). Use pre-warmed (37°C) cell culture media (**30-50 ML**) to recover cryo-preserved cells and when changing media or splitting cells.

Coating of flasks or dishes:
Coat sterile culture dishes or flasks with Gelatin-Based Coating Solution (*Cell Biologics*, Catalog No. 6950) for 2 min and then aspirate the excess solution before seeding cells.

Handling of Arriving Live Cells
When you receive the live cells in a T25 or T75 flask, remove the sticker from the filter cap, and keep the flask with 6-20 ml existing medium in 37°C CO_2 incubator for 1 hour before replacing the desired *Cell Biologics'* cell culture medium. Either split the 95-100% confluent cells from a T25 flask to a T75 flask after 1 hour or let the cells grow in the T25 flask with the desired Medium (such as M1168) for 12 hours before subculturing cells. The recommended split ratio for primary cells is 1:2.

Cell recovery from cryovial:

- Quickly thaw cells in cryo-vial by incubating them in a 37°C water bath for <1 min until there is just a small bit of ice left in the vial.
- Promptly remove the vial and wipe it down with 70% ethanol.
- Transfer cells from the vial to a sterile centrifuge tube. Add 8-10 ml of pre-warmed *Cell Biologics* Cell Culture Medium.
- Flush the vial with an additional 0.5-1 ml of medium to ensure complete transfer of cells to the centrifuge tube.
- Centrifuge cells at 200 g for 5 minutes.
- Aspirate the supernatant and resuspend the cell pellet in 6 ml of *Cell Biologics'* Cell Culture Growth Medium.
- **Recovery cells from cryovial in 10% FBS for the first and second days.**
- **Add resuspended cells into a T25 flask pre-coated with Gelatin-Based Coating Solution (*Cell Biologics,* Catalog No. 6950).**
- **Place the T25 flask in a humidified, 5%-CO_2 incubator at 37°C.**
- **Change culture media the following day to remove non-adherent cells and replenish nutrients.**
- Change cell culture medium every day when cells are >70% confluent.
- Cells should be checked daily under a microscope to verify appropriate cell morphology.

Expansion of cultured primary cells:

- Remove and discard the cell culture media from the flask.
- Flush the adherent layer 2 times using a 5 ml sterile pipette with sterile PBS (1X) without calcium and magnesium to dislodge loosely attached cells and remove fraction.
- Remove and discard the wash solution from the flask.
- **Incubate cells with warm (37°C) 0.25% Trypsin-EDTA solution (*Cell Biologics*, Catalog No. 6914) for 3-5 minutes.** Use 3.0 ml of Trypsin-EDTA solution when collecting cells from a T75 flask, and 2 ml when using a T25 flask. As soon as cells have detached **(the flask may require a few firm gentle taps),** add 8-10 ml of *Cell Biologics*' Cell Culture Medium supplemented with 5-10 % FBS to a T25 or T75 flask (the FBS will neutralize the trypsin).
- Plate cells in fresh flasks or plates precoated with Gelatin-Based Coating Solution in a humidified, 5%-CO_2 incubator at 37°C.
- **Change culture media the following day to remove non-adherent cells and replenish nutrients.**
- Cells should be checked daily under a microscopy to verify appropriate cell morphology.
- Change culture medium every 24-48 hours. Please note that the medium should be changed every day when cells are >70% confluent to remove non-adherent cells and replenish nutrients. Pre-wash cells with 1X PBS 1-2 times whenever replacing the medium.

We recommend splitting primary cells at the follow ratio:

- The recommended split ratio for primary murine cells is 1:2.
- A confluent monolayer of primary cells grown in a T75 flask may be expanded on a 6-well plate ready for use in experiments under the cell culture conditions specified by *Cell Biologics.*

Procedure for Freezing Cells

Materials:

- 1X Phosphate Buffered Saline (PBS-1X)
- 0.25% Trypsin-EDTA (1X) solution (*Cell Biologics,* Catalog No. 6914)
- Tissue Culture Media
- Cold Freezing Media (10% DMSO, 50% FBS and 40% culture medium, Catalog No. 6916, *Cell Biologics*).
- Labeled Cryovials
- Confluent cells

- Remove and discard the cell culture media from the flask.
- Flush the adherent layer with a 5 ml sterile pipette 2 times with sterile PBS (1X) without calcium and magnesium to dislodge loosely attached cells and remove fraction.
- Remove and discard the wash solution from the flask.
- **Incubate cells with warm (37°C) 0.25% Trypsin-EDTA solution (*Cell Biologics*, Catalog No. 6914) for 3-5 minutes.** Use 3.0 ml of 0.25% Trypsin-EDTA solution when collecting cells from a T75 flask, and 2 ml when using a T25 flask. As soon as cells have detached **(the flask may require a few firm gentle taps),** add 10 ml of Cell Culture Medium supplemented with 5-10 % FBS to the flask (the FBS will neutralize the trypsin).
- Centrifuge the cell suspension at 200 g for 5 minutes.
- Remove supernatant with sterile pipette.
- Quickly re-suspend pellet by adding 1 ml freezing media per vial to be frozen.
- Place vials in Nalgene "Mr. Frosty" freezing container containing100% isopropyl alcohol at -70-80 °C for 24 h.
- Transfer vials to liquid N_2 tank for indefinite storage.

We recommend freezing primary cells at the follow ratio:

- A confluent primary endothelial cells grown in a T75 flask may be frozen in 2 cryovials.
- A confluent primary endothelial cells grown in a T25 flask may be frozen in 1 cryovial.

Flow Cytometry in Mouse Primary Endothelial Cell Analysis

General protocol for flow cytometry procedure to use an unconjugated primary antibody (2-step staining)

- **Harvest Mouse Endothelial Cells and keep cells in the cell culture medium (M1168, freshly made Medium, 1-2 weeks) containing 1-10 ng/ml VEGF and 10% FCS for 20 min at 37°C (note: you may let cells over-growing for 24-48h after cells reach confluence; it may take 3-5 days for cell to reach confluence in a T25 flask).**
- Wash the cells 1 time with blocking buffer (1% BSA in 1X PBS with Calcium & Magnesium).
- Adjust Mouse Endothelial Cell suspension to a concentration of at least 0.5 x 10^6 cells/ml in 1-2% BSA in 1X PBS with Calcium & Magnesium.
- Keep cells in blocking buffer for 30 min at RT.
- Add 0.1-10 µg/ml of the primary antibody (UNCONJUGATED). In general, we use 1.0 ug/ml of anti-mouse CD31 antibody, Catalog No. AF3628, Anti-Mouse CD31/PECAM-1 (Polyclonal Goat IgG) from R&D SYSTEMS, INC. or CD31/PECAM-1 (Purified Rat Anti-Mouse CD31, Catalog No. 553370, BD).
- Take 1.0 ul primary antibody from stock solution (0.2mg/ml) into 200 ul sample.
- Incubate cells in eppendorf tubes for at least 30-45 min with gentle shaking at RT.
- Wash the cells two times (1-1.5 ml blocking buffer) by centrifugation at 200 g for 5 minutes and resuspend them in 500 µl.
- Add Second Antibody, Catalog No.: A-11055 (1:200 - 1:400 dilution, The Alexa Fluor® 488 Donkey Anti-Goat IgG (H+L), Invitrogen) and incubate in the dark at room temperature with gentle shaking for 30 minutes.
- Wash the cells 2 times (1-1.5 ml blocking buffer) by centrifugation at 200 g for 5 minutes and resuspend them in 0.4 ml (5 ml Round-Bottom Tube with Cell-Strainer Cap, Catalog No. 352235 BD).
- Keep the cells in the dark on ice and analyze the cells ASAP between 5 min - 2 hours by FACS.

Note:

- You may let cells over-growing for 24-48 h after cells reach confluence before doing any cell testing, cell staining, FACS, or designed experiments.
- Cells can be filtered through a 40 Micron cell strainer (BD 352340) just before passaging cells or doing experiments to remove any clumps of cells and most of the dead/floating large cells.
- Please send us the cell images (>90% confluence) if you have any question or problem with cultured cells.
- Per request, a Certificate of Analysis will be provided for each cell lot purchased.