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Cynomolgus Monkey Primary Small Intestinal Microvascular Endothelial Cells

Catalog No. **MK-6054**

Suggested Medium: Complete Monkey Endothelial Cell Medium – 125 ml
Catalog No. **MK1168**

Product Description

Cynomolgus Monkey Primary Small Intestinal Microvascular Endothelial Cells from *Cell Biologics* are isolated from Cynomolgus monkey small intestinal tissue. Cynomolgus Monkey Primary Small Intestinal Microvascular Endothelial Cells are grown in T25 tissue culture flasks pre-coated with gelatin-based solution for 2 min and incubated in *Cell Biologics'* Culture Complete Growth Medium generally for 3-7 days. Cultures are then expanded. 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 beads pre-coated with secondary antibody.

Product Testing

Cynomolgus Monkey Primary Small Intestinal Microvascular Endothelial Cells from *Cell Biologics* display typical cobblestone with large dark nuclei appearance under light microscopy. Cells are tested for expression of endothelial cell marker using antibody CD31 (Catalog No. ab9498, Abcam), VE-cadherin Antibody (Catalog No. sc-28644, Santa Cruz), or VE-cadherin (Catalog No. ALX-210-232-C100, Enzo Life Sciences, Inc.) by immunofluorescence staining or FACS. Cells are negative for bacteria, yeast, fungi and mycoplasma. Cells can be expanded on a plate ready for experiments under the cell culture conditions specified by *Cell Biologics.* Repeated freezing and thawing of cells is not recommended.

Laboratory Applications

Cynomolgus Monkey Primary Small Intestinal Microvascular Endothelial Cells can be used in assays of cell to cell adhesion, migration, vascular tube formation. 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

Cynomolgus Monkey Primary Small Intestinal Microvascular 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

Appropriate safety procedures should always be used with this material. Although Monkey Primary Small Intestinal Microvascular Endothelial Cells are isolated from normal monkey tissue, 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. The entire text of discussing Biosafety in Microbiological and Biomedical Laboratories, 5th ed. is available online at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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 T75 flask to a 6-well plate for experiment after 1 hour or let the cells grow in the flask with the desired Medium (such as MK1168) 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 150 g for 5 minutes.
- Aspirate the supernatant and resuspend the cell pellet in 6 ml of *Cell Biologics'* Cell Culture Growth Medium.
- **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:

- Flush the adherent layer with a 5 ml sterile pipette 1-2 times to dislodge loosely attached cells.
- Remove and discard the cell culture media from the flask.
- Wash adherent cells 2 times with 10 ml of sterile PBS (1X) without calcium and magnesium to remove nonadherent cells or fraction.
- Remove and discard the wash solution from the flask.
- Incubate cells with warm (37°C) 0.05% Trypsin-EDTA (1X) solution (Invitrogen catalog number 25300) for 1 minute. 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.
- 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 2 times whenever replacing the medium.

We recommend splitting primary cells at the follow ratio:

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

Note:

- You may let cells over-growing for 24-48h 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 or 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 questions or problems with cultured cells.
- Per request, a Certificate of Analysis will be provided for each cell lot purchased.