Rat Brain Microvascular Endothelial Cells

Cat. No. : CP-R108

General Information

Species	Rat/SD rats
Tissue Type	Circulatory system
Tissue	Brain tissue
Cell Type	Endothelial cell
Morphology	Endothelial-like
Growth Properties	Adherent
Storage Conditions	For long-term cryopreservation, cryovials should be stored in liquid nitrogen at −150°C to −196°C. Storage at −80°C is restricted to short-term interim use only.

Culture Conditions and Handling

Complete Medium	Rat Brain Microvascular Endothelial Cell Complete Medium[CM-R108]
Population Doublings	Guaranteed to further expand for 2 population doublings
Subcultivation Ratio	1:2
Dissociation Reagent	Accutase, 0.25% trypsin
Coating Conditions	PLL (0.1 mg/mL) or gelatin coating solution (0.1%)
Medium Renewal	Every 2 to 3 days
Freezing Medium	General Freezing Medium[PB180436]
Incubation Atmosphere	Air, 95%; CO_2, 5%
Temperature	37°C
Instructions	1. Check all containers for leakage or breakage. 2. Remove the frozen cells from the dry ice packaging and immediately transfer them to liquid nitrogen (liquid or vapor phase) for long-term cryopreservation. 3. These cells exhibit relatively strict density dependence. It is recommended to subculture at a 1:2 split ratio when cell density exceeds 90%, and to culture in vessels of the same growth surface area. Subculture at less than 1/2 density is not recommended. At lower densities, cells may exhibit slow growth, morphological changes, and other issues.
Subculturing Procedure	Adherent cell dissociation

Procell system®

Subculturing Procedure

1. Note: Trypsin may damage endothelial cells and cause cell death. Accutase is recommended; prepare the reagent beforehand.
2. Aspirate the culture medium from the T25 flask and wash the cells once with phosphate-buffered saline (PBS).
3-1. Add 1 mL of Accutase solution to fully cover the adherent cells, then incubate the flask at 37°C for 2–5 min. Observe under an inverted microscope until the cells retract and become rounded.
3-2. If Accutase is unavailable, add 1 mL of 0.25% trypsin solution to the T25 flask. Gently rock the flask to allow the trypsin solution to cover the entire bottom surface, then aspirate 500–700 µL of the trypsin solution. Incubate the flask at 37°C for 1–3 min and examine the cells under an inverted microscope until the cells retract and become rounded.
4. Add 5 mL of complete medium to terminate digestion. Gently pipette along the flask wall to dislodge and homogenize the cells into a cell suspension. Seed the suspension into a new T25 flask at the recommended split ratio, and top up with fresh complete medium to a final volume of 5 mL. Incubate the flask in a humidified incubator at 37°C with 5% CO_2 .
5. Once the cells have fully adhered, assess cell viability and confluency under a microscope prior to downstream experiments. Subsequently, refresh the medium with fresh complete medium per the standard cell maintenance protocol.

Background

Rat brain microvascular endothelial cells (*Rattus norvegicus*) are isolated from brain tissue. They are monolayer squamous endothelial cells lining the luminal surface of brain microvessels, with well-developed tight junctions. They are the main component of the blood-brain barrier and express tight junction protein ZO-1. Their core functions include: forming the blood-brain barrier to selectively control the passage of substances into and out of brain tissue; regulating cerebral blood flow; and maintaining the homeostasis of the central nervous system microenvironment. Barrier dysfunction of rat brain microvascular endothelial cells is closely associated with stroke and Alzheimer's disease. In vitro-cultured rat brain microvascular endothelial cells serve as a classic cell model for investigating blood-brain barrier function.

Rat brain microvascular endothelial cells isolated from Procell Laboratory are prepared by combined collagenase-dispase digestion, followed by density gradient centrifugation and selective culture in specialized endothelial cell medium. Each vial contains approximately 5×10^5 cells.

Rat brain microvascular endothelial cells isolated from Procell Laboratory exhibit positive immunofluorescence staining for CD31, with a purity greater than 90%. In addition, the cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi.

Handling Recommendations for Cryopreserved Cells

Preparations Before Thawing

1. Primary cells have high nutritional demands; therefore, prepare complete culture medium in advance.
2. Preheat the complete culture medium to 37°C for 30 minutes.
3. Prepare 9 mL of complete medium within a sterile centrifuge tube to dilute the cryoprotectant present in the frozen cell suspension.
4. Use sterile gloves to protect the cryovial from contact with the water bath water, and preventing cell contamination.

Thawing Procedure

1. Retrieve the cryovial containing frozen cells from liquid nitrogen storage. Place it in sterile gloves and immediately submerge it in a 37°C water bath.
2. Thaw the cells rapidly (<1 minute) by gently swirling the vial in the 37°C water bath until only a small ice crystal remains.
3. Transfer the vial to a laminar flow hood. Wipe the exterior of the vial with 75% ethanol before opening.
4. Transfer the thawed cells dropwise into a preheated 9 mL centrifuge tube containing complete medium.
5. Centrifuge the cell suspension at approximately 250 × g for 5 minutes. For cells characterized by low density and small volume, such as lymphocytes and suspended cells, the centrifugation speed can be appropriately increased to 400 × g for 8 minutes.
6. After centrifugation, discard the supernatant and retain the cell pellet.
7. Gently resuspend the cells in complete growth medium, then transfer them to an appropriate culture vessel and place it in the recommended culture environment.

Notes

1. The entire recovery process should be completed as quickly as possible.
2. Select the culture vessel size based on the number of cells in the cryovial. Procell single cryovials are recommended for resuspension in 6 cm dishes or T25 flasks.
3. Minimize the time thawed cells are kept at room temperature. DMSO must be immediately diluted or removed by centrifugation.

Precautions After Receipt of Frozen Cells

1. Upon unpacking, inspect the condition of the frozen cells and dry ice, and take photographs immediately. The following after-sales service will be provided based on these photographs, including assessment of the remaining dry ice, verification that the cryovial was fully buried in dry ice, and evaluation of whether the cells thawed and refrozen during transit.
2. Upon receipt, the cells should be transferred to liquid nitrogen immediately or directly resuscitated. If liquid nitrogen is unavailable, the cells can be temporarily stored at -80°C, however, the storage period should be limited to less than one week whenever possible. Prolonged storage at -80°C may gradually reduce post-thaw cell viability, and the extent of this viability loss is unpredictable.
3. Ensure that the operator has sufficient knowledge and experience in cell culture, and the laboratory is equipped with essential instruments, including a biosafety cabinet, CO_2 incubator, inverted microscope, centrifuge, water bath. Carefully review the cell instruction manual to understand key cell characteristics including growth properties (adherent or suspension), morphology, basal medium requirements, serum concentration, cytokine supplementation, subcultivation ratio, medium renewal schedule.
4. After resuscitation, observe the cells under a microscope and record the cell status by taking photographs (1-3 images each at 100× and 200× magnification for 3 consecutive days). These images will serve as supporting documentation for follow-up services. In addition, a small aliquot of cells may be used to assess cell viability by automated cell counting or trypan blue staining.

Note: Cells should not be observed too frequently within the first 24 hours after resuscitation, as this may affect cell growth or adherence. Observation once per day is sufficient.

5. Primary cells have a limited number of passages; it is recommended that they be used as soon as possible.