bEnd.3 [BEND3] Cell Line

Cat. No. : CL-0598

Origin and General Characteristics

Cell Name	bEnd.3 [BEND3]
Organism	Mouse
Age	6W
Sex	Sex unspecified
Tissue	Brain
Morphology	Epithelial-like
Growth Properties	Adherent
Background	bEnd.3 [BEND3] is a mouse brain microvascular endothelial cell line isolated from endothelioma-bearing mouse brain tissue and immortalized via retroviral transduction of polyomavirus middle T antigen. bEnd.3 cells display canonical endothelial phenotypes, such as von Willebrand factor expression and the capacity to internalize low-density lipoprotein (LDL). bEnd.3 cells constitutively produce intercellular adhesion molecule 1 (ICAM-1), and ICAM-1 expression is further elevated following exposure to LPS, IL-1, and TNF-α. Early-passage bEnd.3 cells express surface MAdCAM-1 under basal conditions, yet this marker disappears beyond passage 30. bEnd.3 cells also constitutively express vascular cell adhesion molecule 1 (VCAM-1) prior to passage 30, with VCAM-1 expression undetectable at later passages. Tumor necrosis factor-α (TNF-α) triggers P-selectin expression in bEnd.3 cells, and this inductive effect is more pronounced in cells cultured past passage 30. bEnd.3 cells serve as a standard in vitro model for blood-brain barrier and neurovascular biology investigations.
Biosafety Level	BSL-1

Culture Conditions and Handling

Complete Medium	DMEM[PM150210]+10% Nutrient+1% Supplement
Subcultivation Ratio	1:2-1:4
Medium Renewal	2 to 3 times per week
Dissociation Duration	3-8 min
Incubation Atmosphere	Air, 95%; CO_2, 5%
Temperature	37°C
Freeze Medium	General Freezing Medium[PB180436]
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.

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. At low density, cells have irregular morphology, low cell number, and a large spreading area. At high density, morphology becomes more uniform and cell bodies are dense.
4. Cell proliferation is relatively slow. A 1:2 split ratio is recommended for the first subculture. After cells stabilize, the subculture split ratio should also not exceed 1:4.
5. Monitor the color of the culture medium; a purplish color will cause increased cell floating.
6. Dissociation time varies with cell growth duration and cell density. It should be determined based on cell condition during dissociation. Terminate dissociation only when cells can be dislodged.

Subculturing Procedure

1. Remove the culture medium from the T25 cell culture flask.
2. Add approximately 2 mL of PBS. Gently tilt the flask side to side until the PBS covers the entire bottom, then aspirate and discard the PBS.
3. Add 1 mL of 0.25% trypsin solution (containing EDTA). Gently tilt the flask side to side until the trypsin solution covers the entire bottom of the flask.
4. Incubate the cells at 37°C. Observe the cells under an inverted microscope and terminate digestion once the cells round up and detach. To avoid clumping, do not agitate the cells by tapping or shaking the flask during detachment.
5. Add 3 mL of complete culture medium to terminate digestion and disperse into a single cell suspension.
6. Collect the cell suspension and centrifuge at 1200 rpm (approximately 250 ×g) for 3 minutes. Carefully aspirate and discard the supernatant.
7. Add fresh complete culture medium, pipette gently several times to resuspend the cells, and seed them at the appropriate ratio into a new culture flask. Loosen the cap or use a vented cap for incubation.

Special Features of the Cell Line

Antigen Expression

ICAM-1 +; VCAM-1 +; MAdCAM-1 +

Genes Expressed

von Willebrand factor,ICAM-1 +; VCAM-1 +; MAdCAM-1 +,The endothelial nature of these cells was confirmed by the observed expression of von Willebrand factor and uptake of fluorescently labeled low density lipoprotein (LDL). The expression of Peyer's Patch high endothelial receptor for lymphocytes, the mucosal vascular addressin (MAdCAM-1) and E-selectin can be induced on bEnd3.

Handling Recommendations for Cryopreserved Cells

Preparations Before Thawing

1. Prepare the recommended complete culture medium and preheat it to 37°C for 30 minutes.
2. Prepare 9 mL of complete medium within a sterile centrifuge tube to dilute the cryoprotectant present in the frozen cell suspension.
3. 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 biosafety cabinet. 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.
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 T25 flasks or 10 cm dishes.
3. Minimize the time thawed cells are kept at room temperature. Cryoprotectant 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 two weeks 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. After sufficient expansion, the cells should be cryopreserved as soon as possible to ensure availability of backup stocks for future experiments or in case of unexpected experimental failure. Cells in the logarithmic growth phase should be selected for cryopreservation. After cryopreservation, it is recommended to resuscitate one vial to verify post-thaw viability and confirm successful cryopreservation.