

## Data Sheet

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|                           |   |
|---------------------------|---|
| <b>BCRJ Code:</b>         | 0433  |
| <b>Cell Line:</b>         | bEND.3  |
| <b>Species:</b>           | Mus musculus  |
| <b>Vulgar Name:</b>       | Mouse; Balb/C   |
| <b>Tissue:</b>            | Brain; Cerebral cortex  |
| <b>Cell Type:</b>         | Epithelial  |
| <b>Morphology:</b>        | Endothelial   |
| <b>Disease:</b>           | Endothelioma  |
| <b>Growth Properties:</b> | Adherent  |
| <b>Age/Ethnicity:</b>     | 6 Week /  |
| <b>Derivation:</b>        | The cells were transformed by infection with the NTKmT retrovirus vector that expresses polyomavirus middle T antigen.  |
| <b>Applications:</b>      | The cells were transformed by infection with the NTKmT retrovirus vector that expresses polyomavirus middle T antigen. 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). |
| <b>Products:</b>          | Antigen expression: ICAM-1 +; VCAM-1 +; MAdCAM-1 + Genes expressed: von Willebrand factor-; ICAM-1+; VCAM-1+; MAdCAM-1+ ; The expression of Peyer's Patch high endothelial receptor for lymphocytes, the mucosal vascular addressin (MAdCAM-1) and E-selectin can be induced on bEnd.             |
| <b>Biosafety:</b>         | 2   |

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### Additional Info:

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 bEnd.3 cells by cytokines and lipopolysaccharide (LPS). This induction by Tumor Necrosis Factor alpha (TNF alpha), interleukin 1 (IL-1) or LPS is concentration and time dependent. MAdCAM-1 is expressed on the surface of unstimulated bEnd.3 cells at early passages but not at passages greater than 30. Intracellular adhesion molecule 1 (ICAM-1) is constitutively expressed on the cells, and expression is increased by treatment with LPS, IL-1 and TNF alpha. Vascular cell adhesion molecule 1 (VCAM-1) is constitutively expressed on the cells at early passages but not at passages over 30. P-selectin can be induced on bEnd.3 cells by Tumor Necrosis Factor alpha (TNF alpha) at both early and late passages but expression is greater at passages over 30.

### Culture Medium:

Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose and fetal bovine serum to a final concentration of 10%.

### Subculturing:

Remove and discard culture medium. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.03% (w/v) EDTA solution to remove all traces of serum which contains trypsin inhibitor. Add 1.0 to 2.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer just begins to detach. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels.

### Subculturing Medium Renewal:

Every 2 to 3 days

### Subculturing Subcultivation Ratio:

1:6 to 1:10, Split sub-confluent cultures (70-80%), seeding at 2-4x10,000 cells/cm<sup>2</sup>

### Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO<sub>2</sub>), 5% Temperature: 37°C

### Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

## Thawing Frozen Cells:

**SAFETY PRECAUTION:** It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

**NOTE:** It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

## References:

Montesano R, et al. Increased proteolytic activity is responsible for the aberrant morphogenetic behavior of endothelial cells expressing the middle T oncogene. *Cell* 62: 435-445, 1990. PubMed: 2379237 Sikorski EE, et al. The Peyer's patch high endothelial receptor for lymphocytes, the mucosal vascular addressin, is induced on a murine endothelial cell line by tumor necrosis factor- $\alpha$  and IL-1. *J. Immunol.* 151: 5239-5250, 1993. PubMed: 7693807 Williams RL, et al. Embryonic lethality and endothelial tumors in chimeric mice expressing polyoma virus middle T oncogene. *Cell* 52: 121-131, 1988. PubMed: 3345558

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