Bovine Brain Microvascular Endothelial Cell System, bMVEC-B
A Model of the Blood Brain Barrier

Introduction
Clonetics™ Bovine Brain Microvascular Endothelial Cell System contains cryopreserved primary microvascular cells derived from bovine brain tissue and an optimized medium system for the establishment of a confluent monolayer. The model can be used to study active and passive transport of drugs across the blood brain barrier, brain endothelial cell tight junctions and for the study of the basic biology of brain microvascular endothelial cells.

Clonetics™ bMVEC-B System is functional, consistent, convenient and easy to use, allowing the researcher to focus on results. The endothelial cells express tight junctions (positive for the proteins ZO-1 and Claudin-1) and transport characteristics (P-GP function) found in the blood brain barrier. Clonetics™ bMVEC-B cells show lot-to-lot consistency in sucrose permeability studies. Cryopreserved bMVEC-B cells are provided complete with an optimized medium system and comprehensive instructions.

Cell System Components
- Bovine Brain Microvascular Endothelial Cells, bMVEC-B (Cryopreserved)
- EMVB BulletKit™ containing EBM™-2 Basal Medium and a SingleQuots™ Kit containing the following: Ascorbic Acid, 1 X 0.5 ml; β-ECGF, 1 X 0.5 ml; Horse Serum, Platelet Poor, 1 X 50 ml; Heparin, 1 X 1.0 ml; Penicillin/Streptomycin/Fungizone, 1 X 5.0 ml.

Characterization of Cells
Routine characterization of bMVEC-B includes:
- Positive testing for the drug transporter P-glycoprotein function by calcein AM efflux assay using verapamil hydrochloride as a P-GP inhibitor
- Acetylated LDL uptake

Performance
Recommended seeding density for 24 well plates 20,000 cells/cm²
Recommended seeding density for membrane culture inserts 50,000 cells/cm²
Typical time from seeding to confluent barrier 8 - 11 days

Calcein AM Efflux for P-GP Function Using Verapamil HCl as a P-GP inhibitor
Permeability of [14C] Sucrose Across Bovine Brain Microvascular Endothelial Cells Cultured for 10 Days

Permeability Coefficient (cm/min x 10^-4)

<table>
<thead>
<tr>
<th>Lot</th>
<th>Permeability Coefficient (cm/min x 10^-4)</th>
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<tbody>
<tr>
<td>Lot #1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Lot #2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Lot #3</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Lot #4</td>
<td>5 ± 1</td>
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</tbody>
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Quality Control
All bMVEC-B cells are characterized for functionality (see Characterization of Cells). bMVEC-B cells are cultured without antimicrobial agents and assayed to ensure the absence of microbial contamination after cryopreservation. After recovery from liquid nitrogen, cells are tested for viability, proliferative capacity, mycoplasma, yeast, fungus, and bacteria. Certificates of Analysis (COA) for each cell strain are shipped with each order. COA for all other products are available upon request. Lonza can provide non-routine performance and quality testing to meet your specifications for an additional fee.

Ordering Information

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>AC-2509</td>
<td>bMVEC-B, Bovine Brain Microvascular Endothelial Cells (Cryopreserved)</td>
<td>≥750,000 cells/amp</td>
</tr>
<tr>
<td>AC-3103</td>
<td>EMVB BulletKit™ – Consists of EBM™-2 Basal Medium and EMVB SingleQuots™ to formulate media for the growth of bMVEC-B</td>
<td>500 ml</td>
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<tr>
<td>CC-3156</td>
<td>EBM™-2 Endothelial Cell Basal Medium-2 (no growth factors)</td>
<td>500 ml</td>
</tr>
<tr>
<td>AC-4207</td>
<td>EMVB SingleQuots™, (growth factors and supplements)</td>
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<tr>
<td>AA-1035</td>
<td>Comprehensive instruction sheet</td>
<td></td>
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Cells should be plated onto tissue cultureware coated with rat tail collagen type I or onto membrane inserts coated with rat tail collagen type I and overcoated with fibronectin. Tissue culture plasticware, membrane inserts, rat tail collagen type I and fibronectin are not provided but are available commercially.

When placing an order or for technical service, please refer to the product numbers and descriptions listed above. To obtain a price list, additional information or technical service you may contact Lonza by telephone, fax or mail.

Product Warranty
CULTURES HAVE A FINITE LIFESPAN IN VITRO.
Lonza warrants its cells only if Clonetics™ Media are used, and the recommended protocols are followed. Cryopreserved bovine brain microvascular endothelial cells are assured to be viable and functional when thawed and maintained properly.

References:

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures.

WARNING: CONTAINS BOVINE SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIONOUS. PREPARED FROM U.S. SOURCE BOVINE BRAIN. Not tested for bovine viruses or BSE. Products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. If you require further information, please contact your site Safety Officer or Scientific Support.