





Product Sheet

Microvascular Endothelial Cell Growth Kit-BBE (ATCC® PCS-110-040™)

Please read this FIRST



Storage Temp.
-20°C (or -70°C for long-term storage)



Biosafety Level
1

Description

Product Description: Microvascular Endothelial Cell Growth Kit-BBE (ATCC® PCS-110-040) and Microvascular Endothelial Cell Growth Kit-VEGF (ATCC® PCS-110-041) each contain components that when added to Vascular Cell Basal Medium (ATCC® PCS-100-030) create a complete ATCC® Primary Cell Solution™ culture environment for microvascular endothelial cells derived from normal human tissue (e.g., Primary Dermal Microvascular Endothelial Cells; Normal, Human, Neonatal, ATCC® PCS-110-010). Your experimental design will dictate which Microvascular Endothelial Cell Growth Kit should be used. Use of the Microvascular Endothelial Cell Growth Kit-VEGF (ATCC® PCS-110-041) will support a faster rate of proliferation because of the presence of several purified human recombinant (rh) growth factors (rh_VEGF, rh_EGF, rh_FGF basic and rh_IGF-1) combined with heparin and hydrocortisone. Use of the Microvascular Endothelial Cell Growth Kit-BBE (ATCC® PCS-110-040), which contains Bovine Brain Extract (BBE), is recommended if a less defined cell culture medium is desired.

Volume: 1 kit

Directions for Use

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. Store the basal medium at 2°C to 8°C and the growth kit(s) at either -20°C in a freezer that is not self-defrosting or at -70°C for long-term storage. If thawed upon arrival, the growth kit should be stored at 2°C to 8°C and added to the basal medium within 72 hours of receipt.

Preparation of Complete Growth Media

1. Obtain one growth kit from the freezer; make sure that the caps of all components are tight.
2. Thaw the components of the growth kit just prior to adding them to the basal medium. It is necessary to warm the L-glutamine component in a 37°C water bath and shake to dissolve any precipitates prior to adding to the basal medium.
3. Obtain one bottle of Vascular Cell Basal Medium (475 mL) from cold storage.
4. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by spraying them with 70% ethanol.
5. Using aseptic technique, and working in a laminar flow hood or biosafety cabinet transfer the volume of each growth kit component, as indicated in Table 1 or 2, to the bottle of basal medium using a separate sterile pipette for each transfer.

Table 1. If using the Microvascular Endothelial Cell Growth Kit-BBE (ATCC® PCS-110-040), add the indicated volume for each component:

| Component | Volume | Final Concentration |
|------------------------------|---------|---------------------|
| Bovine Brain Extract (BBE) | 1.0 mL | 0.2% |
| rh EGF | 0.5 mL | 5 ng/mL |
| L-glutamine | 25.0 mL | 10 mM |
| Heparin sulfate | 0.5 mL | 0.75 Units/mL |
| Hydrocortisone hemisuccinate | 0.5 mL | 1 µg/mL |
| Fetal Bovine Serum | 25.0 mL | 5% |
| Ascorbic acid | 0.5 mL | 50 µg/mL |

Table 2. If using the Microvascular Endothelial Cell Growth Kit-VEGF (ATCC® PCS-110-041), add the indicated volume for each component:

| Component | Volume | Final Concentration |
|------------------------------|---------|---------------------|
| rh VEGF | 0.5 mL | 5 ng/mL |
| rh EGF | 0.5 mL | 5 ng/mL |
| rh FGF basic | 0.5 mL | 5 ng/mL |
| rh IGF-1 | 0.5 mL | 15 ng/mL |
| L-glutamine | 25.0 mL | 10 mM |
| Heparin sulfate | 0.5 mL | 0.75 Units/mL |
| Hydrocortisone hemisuccinate | 0.5 mL | 1 µg/mL |
| Fetal Bovine Serum | 25.0 mL | 5% |
| Ascorbic acid | 0.5 mL | 50 µg/mL |

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Antimicrobials and phenol red are not required for proliferation but may be added if desired. The recommended volume of each **optional** component to be added to the complete growth media is summarized in Table 3.

Table 3. Addition of Antimicrobials/Antimycotics and Phenol Red (Optional)

| Component | Volume | Final Concentration |
|---|--------|---|
| Gentamicin-Amphotericin B Solution | 0.5 mL | Gentamicin: 10 µg/mL Amphotericin B: 0.25 µg/mL |
| Penicillin-Streptomycin-Amphotericin B Solution | 0.5 mL | Penicillin: 10 Units/mL Streptomycin: 10 µg/mL Amphotericin B: 25 ng/mL |
| Phenol Red | 0.5 mL | 33 µM |

6. Tightly cap the bottle of complete growth medium and swirl the contents gently to assure a homogeneous solution. Do not shake forcefully to avoid foaming. Label and date the bottle.
7. Complete growth media should be stored in the dark at 2°C to 8°C (do not freeze). When stored under these conditions, complete media is stable for 30 days.

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