

BIOCOAT® Endothelial Cell Growth Environment

Catalog No. 355053/355054

Lot Number:

Instructions for Use

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

Use restriction for Europe and the United Kingdom: This product may only be used as an *in-vitro* laboratory reagent. This product and its residue must not be allowed to come into contact with ruminating animals or swine.

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INDEX

INTENDED USE	2
SUMMARY	2
MATERIALS PROVIDED	3
MATERIALS REQUIRED BUT NOT SUPPLIED	3
PRECAUTIONS	4
PROCEDURE FOR USE	4
1.0 Reconstitution of E-STIM□ Culture Medium Supplements	4
2.0 Supplementation of E-STIM□	5
3.0 Reconstitution and Storage of Trypsin Inhibitor	5
4.0 Seeding of Cells	5
5.0 Feeding the Cultures	6
6.0 Trypsinization of Cultures	6
TYPICAL RESULTS	7
ORDERING INFORMATION	8
TECHNICAL SERVICES	8
REFERENCES	8

INTENDED USE

The BIOCOAT® Endothelial Cell Growth Environment is an integrated system designed to promote rapid growth of monolayers of endothelial cells in vitro.

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SUMMARY

The BIOCOAT® Endothelial Cell Growth Environment is a system containing the materials necessary for the establishment, growth, and support of primary monolayer cultures and subcultures of endothelial cells in low-serum conditions. This system has been shown to promote rapid growth (at least six fold increase in cell number over five days) of endothelial cells from a variety of sources, including human umbilical vein (HUVEC) pulmonary artery (HPAEC) and aorta (HAEC), as well as fetal bovine heart (FBHEC).

The BIOCOAT® Endothelial Cell Growth Environment (355053) contains:

- a) E-STIM™ Endothelial Cell Culture Medium, a low-serum medium that has been optimized for the growth of endothelial cell cultures;
- b) Epidermal Growth Factor (EGF) and Endothelial Cell Growth Supplement (ECGS), supplements for the E-STIM medium stimulating the rapid growth of endothelial cells in vitro;
- c) BIOCOAT® Type I Collagen (rat tail) Cellware (not included in 355054) providing a culture substrate shown to be produced in vivo in regions of angiogenesis and endothelial sprout formation¹, and to promote attachment and stimulate mitosis in endothelial cells in vitro^{3,5} (see Figure 1); and
- d) Soybean Trypsin Inhibitor, to inactivate trypsin used for subculturing cells under low-serum conditions.

Endothelial Cells grown using this BIOCOAT® Growth Environment may be subcultured according to routine procedures, seeded into bioassays or fermentation systems, used for isolation of cellular products or components, or used in a variety of other applications employing endothelial cells.

EFFECTS OF EXTRACELLULAR MATRIX ON GROWTH OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

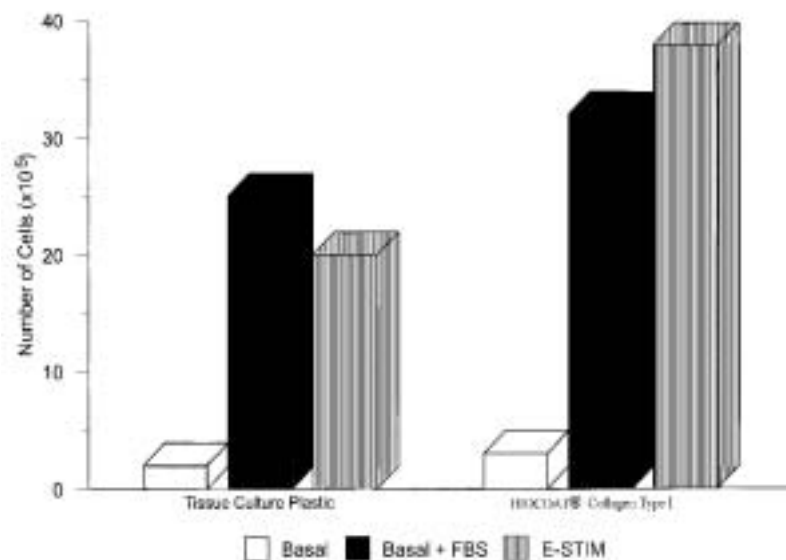


Figure 1. 5×10^5 HUVEC were plated into 75 cm^2 tissue culture flasks or 75 cm^2 BIOCOAT® Collagen Type I flasks. Cells were grown for five days in basal medium with or without 20% FBS or in E-STIM™ and then harvested for counting.

MATERIALS PROVIDED

- E-STIM™ Endothelial Cell Culture Medium (MCDB 131 containing hydrocortisone, heparin and 2% FBS), 500 ml.
- EGF, receptor grade, 5 μg (lyophilized)
- ECGS, 100 mg (lyophilized)
- BIOCOAT® Type I Collagen (rat tail) Cellware (included only in P/N 355053)
- Soybean Trypsin Inhibitor, 50 mg (lyophilized)

MATERIALS REQUIRED BUT NOT SUPPLIED

- 70% ethanol/water solution
- FALCON® sterile pipettes: 10 ml, 5 ml, 1 ml
- FALCON® sterile centrifuge tubes: 50 ml, 15 ml
- Trypsin-EDTA solution (for subculturing)
- Laminar flow tissue culture hood
- Humidified tissue culture incubator, 37°C, 5% CO₂

PRECAUTIONS

- a) Storage: Materials should be stored at 2-8°C in original packaging with box lid closed to avoid possible deterioration of culture medium caused by fluorescent lighting. **DO NOT FREEZE.**
- b) Reconstitution: The components of this BIOCOAT® Growth Environment have been tested and found negative for the presence of bacteria, fungi, and mycoplasma, and E-STIM™ contains < 0.1 endotoxin units/ml (LAL assay). Culture medium supplements should be added under aseptic conditions; there is no need for sterile filtration of the supplemented medium, which may adversely affect its bioactivity. **NOTE: The E-STIM medium does not contain antibiotics. If this product is to be used with primary cells, the addition of antibiotics is recommended.**
- c) Use of Supplemented Medium: E-STIM Endothelial Cell Culture Medium must be supplemented with 5 µg of EGF and 100 mg of ECGS (provided) prior to use. After supplementation, E-STIM is stable for 6 weeks when stored at 4°C in its original bottle. For use, only as much medium as is immediately necessary should be warmed to 37°C in a separate container (e.g. 50 ml tube) immediately prior to use -- **avoid repeated warming/cooling cycles of the stock of supplemented medium, as this may adversely affect its bioactivity.**
- d) Use of BIOCOAT® Cellware: Endothelial Cells may be seeded into BIOCOAT® Collagen I Cellware according to standard procedures. When cells reach desired density, they may be trypsinized off of the substrate using standard trypsinization protocols (see below). Upon treatment with trypsin, the BIOCOAT® Cellware should be discarded and should not be used for seeding or subculturing of cells.

California Proposition 65 Notice

WARNING: This product contains a chemical known to the state of California to cause birth defects and/or other reproductive harm.

Component: Streptomycin Sulfate

PROCEDURE FOR USE

NOTE: All procedures should be performed under strict aseptic conditions. The E-STIM™ medium does not contain antibiotics. If this product is to be used with primary cells, the addition of antibiotics is recommended.

1.0 Reconstitution of E-STIM Culture Medium Supplements

- 1.1 EGF: Disinfect the vial of EGF and the bottle of E-STIM culture medium with 70% ethanol. In the laminar flow hood, open the vial and reconstitute the lyophilized EGF with 1 ml of E-STIM. Recap the vial and mix by inverting several times, until EGF is completely resuspended.
- 1.2 ECGS: Disinfect the vial of ECGS with 70% ethanol. Open the vial and reconstitute the lyophilized ECGS with 10 ml of E-STIM. Recap the vial and mix by inverting several times, until the ECGS is completely resuspended.

2.0 Supplementation of E-STIM□

- 2.1 Using a 1 ml pipette, aseptically add the solution from the EGF vial into the bottle of E-STIM medium. Rinse the EGF vial twice with 1 ml each of E-STIM, and add the rinses to the E-STIM bottle.
- 2.2 Using a 10 ml pipette, aseptically add the solution from the ECGS vial into the bottle of E-STIM medium. Rinse the ECGS vial twice with 5 ml each of E-STIM, and add the rinses to the E-STIM bottle.
- 2.3 Recap the E-STIM™ bottle and gently mix by inverting several times. **DO NOT SHAKE MEDIUM VIGOROUSLY - FOAMING INDICATES PROTEIN DENATURATION.** Record date of supplementation on the bottle label, and store supplemented medium at 4°C until use. **Expiration Date: after supplementation, 6 weeks at 4°C.**

3.0 Reconstitution and Storage of Trypsin Inhibitor

- 3.1 Disinfect the vial of trypsin inhibitor using 70% ethanol. Aseptically add 10 ml of supplemented E-STIM to the vial of trypsin inhibitor. Recap the vial and invert several times to resuspend; remove the solution into a sterile 50 ml tube.
- 3.2 Rinse the trypsin inhibitor vial four times with 10 ml each of E-STIM; add the rinses into the 50 ml tube from step 3.1. Cap the tube and invert several times until the trypsin inhibitor is completely solubilized.
- 3.3 Store the trypsin inhibitor at -20°C in working aliquots (5-10 ml) until use.

4.0 Seeding of Cells

- 4.1 Warm a volume of E-STIM™ medium in a 37°C water bath, sufficient to seed desired amount of BIOCOAT® Cellware (see Table 1 for recommended volumes). **Please see note in PRECAUTIONS section regarding warming of medium.**
- 4.2 Seed endothelial cells into BIOCOAT® Cellware at a concentration of $3.3 - 6.7 \times 10^3$ cells/cm² (see Table 1 for surface areas of BIOCOAT® Cellware). **Note: Higher seed densities may be used, but may cause clumping of cells on growth surface; lower seed densities may cause sparse plating and decrease growth rate.**

- 4.3 Gently disperse cells over growth surface and place BIOCOAT® Cellware into incubator.

TABLE 1. SURFACE AREAS AND SEEDING VOLUMES FOR BIOCOAT® CELLWARE

Cat. #	BIOCOAT® Cellware Configuration	Surface area cm²/well	Recomm. Vol. of E-STIM□/ well
3053-06	6-well plates	10.80	2.0 ml
3053-12	12-well plates	5.70	1.5 ml
3053-24	24-well plates	2.60	1.0 ml
3053-48	48-well plates	1.46	0.5 ml
3053-96	96-well plates	0.84	0.2 ml
3053-35	35 mm dishes	10.80	2.0 ml
3053-60	60 mm dishes	28.30	5.0 ml
3053-10	100 mm dishes	58.20	10.0 ml
355053	T-75 flasks	75.00	20.0 ml

5.0 Feeding the Cultures

- 5.1 Cultures should be fed every 2-3 days by complete medium exchange (remove spent medium and add an appropriate volume (see Table 1) of fresh E-STIM™). If higher seed densities than those recommended in step 4.2 are used, more frequent feeding may be required.
- 5.2 Cultures should be fed regularly until the desired degree of confluence is reached, at which point cells may be trypsinized for subculture, cryopreservation, or other uses. **NOTE: Cultures should not be allowed to grow to > 80% confluence, as this decreases plating efficiency and growth rate upon subculturing.**

6.0 Trypsinization of Cultures

- 6.1 Thaw one aliquot of trypsin inhibitor (see step 3.0) for each BIOCOAT® plate, dish or flask to be trypsinized.
- 6.2 Disinfect outside of BIOCOAT Cellware with 70% ethanol. Allow ethanol to evaporate before opening cellware in laminar flow hood.
- 6.3 Remove and discard culture medium from cellware. Rinse monolayer with trypsin, and immediately aspirate and discard the rinse.
- 6.4 Add sufficient trypsin to the cellware to cover the monolayer; incubate cellware at 37°C. Cells should detach from growth surface within 5-7 minutes; if cells remain attached after this time, the trypsin is likely to be insufficiently active -- repeat procedure once with fresh trypsin solution.

- 6.5 Once cells have detached, immediately add an equal volume of trypsin inhibitor to the cellware. Triturate contents several times, rinsing the growth surface to completely resuspend cells.
- 6.6 Remove cellware contents into a sterile centrifuge tube. Pellet cells at 500 x g for five minutes.
- 6.7 Aspirate and discard supernatant; resuspend pellet in 10 ml of E-STIM™ and repeat centrifugation.
- 6.8 Cells may be used for subculturing (follow step 4.0), seeding into bioassay, or other applications.

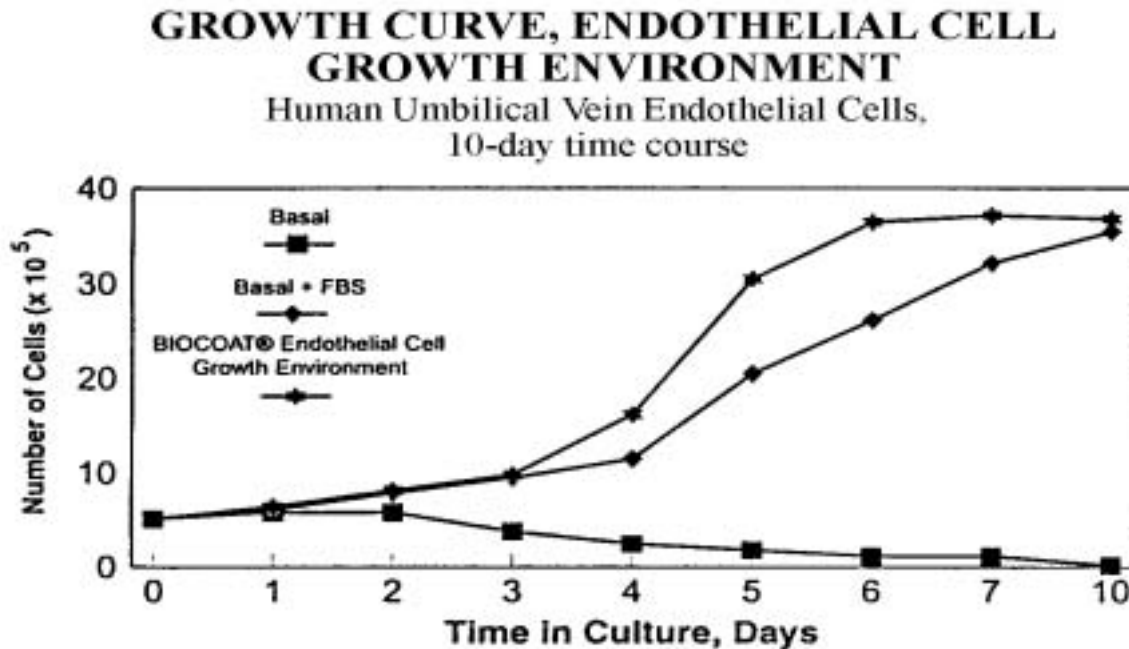


Figure 2. 5×10^5 HUVEC were plated to 75 cm^2 tissue culture flasks in basal medium with or without 20% FBS, or into the BIOCOAT® Endothelial Cell Growth Environment. Cells were harvested daily for counting. Each point represents the mean of three determinations.

TYPICAL RESULTS

When used according to the above procedure, the BIOCOAT® Endothelial Cell Growth Environment should provide the following results:

- a) > 90% attachment of cells to substrate within 1-2 hours of seeding;
- b) > 90% of attached cells showing spreading within 2-4 hours of seeding;
- c) mitotic cells observed within 24-36 hours of seeding;

- d) active growth of cells to 60-80% confluence (6-8 population doublings) within 5-6 days (see growth curve, Figure 2); and
- e) cobblestone or elongated cell morphology, typical of endothelial cells grown on type I collagen.

STABILITY

The components of this Growth Environment are stable for a minimum of 3 months from day of shipment when stored at 2-8°C. Upon supplementation with EGF and ECGS, the E-STIM™ culture medium is stable for 6 weeks when stored at 2-8°C under subdued lighting conditions (see **PRECAUTIONS**).

ORDERING INFORMATION

To order, call our Customer Service Department at (781) 275-0004 or (800) 343-2035 (within the United States, except Massachusetts), telefax (781) 275-0043, telex (170) 326-7601.

TECHNICAL SERVICES

For technical information, please call our Technical Services Department at (781) 275-0004 ext. 389.

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