Corning® BioCoat™ Angiogenesis System-Endothelial Cell Tube Formation

Catalog No. 354149, 354150

Guidelines for Use

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For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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INTENDED USE

The Corning® BioCoat™ Angiogenesis System: Endothelial Cell Tube Formation provides an *in vitro* assay system that allows assessment of a number of cellular events such as attachment, migration, invasion and differentiation in the angiogenesis process as well as the modulation of these events by anti-angiogenic agents. The Corning BioCoat Angiogenesis System offers a tube formation assay in 96-well format and a process of assaying for endothelial cell tube formation and its modulation in a high throughput manner. The system is optimized to allow rapid data collection when using automated image acquisition hardware and data processing software. The Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation consists of a Falcon® 96-well Black/Clear Plate and Corning Matrigel® Matrix optimized for endothelial cell tube formation. Quantification of the extent of tube formation is achieved by digitizing fluorescent tube images followed by measuring tube length using MetaMorph® Software. The Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation is a robust and automation-friendly system designed to enable rapid assessment of angiogenesis modulating agents/molecules on endothelial cell tube formation in a high-throughput fashion.

MATERIALS PROVIDED

- Catalog # 354149 packaged as a 1 plate pack of Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation containing a Falcon 96-well Black/Clear Plate coated with Corning Matrigel Matrix and lid
- Catalog # 354150 packaged as a 5 plate pack of Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation containing five Falcon 96-well Black/Clear Plates coated with Corning Matrigel Matrix and lids

RELATED PRODUCTS

- Endothelial cell culture medium, e.g., EGM-2 MV (Clonetics, Cat. No., CC-4147)
- Fetal bovine serum or appropriate Growth Factor as an angiogenesis stimulator
- Hanks' balanced salt solution (HBSS), e.g., Invitrogen, Cat. No. 14025-092.
- Fluorophore, *e.g.*, Corning Calcein AM Fluorescent Dye, Catalog No. 354216 (50 μg vial)
- DMSO (for calcein AM)
- Humidified tissue culture incubator, 37°C, 5% CO_2 atmosphere
- Endothelial cells such as Human Microvascular Endothelial Cells and Human Umbilical cord endothelial cells
- Sterile blunt-end or tissue forceps
- Laminar flow tissue culture hood
- Automated imager, fluorescence microscope, tube quantification software

SAFETY RECOMMENDATION: Handle in accordance with good industrial hygiene and laboratory safety practices

PRECAUTIONS

- **Storage: Materials should be stored at -20°C in the original packaging. DO NOT STORE IN FROST-FREE OR -70°C FREEZER.**
- **All Procedures should be performed under aseptic conditions except where indicated.**
- **Do not stack plates during thawing or short incubations.**

California Proposition 65 Notice

WARNING: This product contains a chemical known to the state of California to cause cancer.

Component: **Chloroform**

GENERAL USE

The Corning® BioCoat™ Angiogenesis System: Endothelial Cell Tube Formation is evaluated and optimized for tube formation using HMEC-1 cells, a SV40 Large T antigen immortalized human microvascular endothelial cell line. Image acquisition and quantification of endothelial cell tube formation (fluorescently labeled tube) can be achieved by using MetaMorph® software coupled with an automated imager. In the following procedure, assay conditions and cell labeling for quantification of endothelial cell tube formation have been optimized to maximize the fluorescent signal while minimizing the cytotoxic effects of calcein AM on HMEC-1 cells. Results may vary depending upon the cells, dye used and the specific experimental conditions, particularly those relating to medium, dye concentration, incubation time, cell seeding density, and angiogenesis stimulators. Individual researchers should optimize conditions for their system.

1.0 Thawing and polymerization

1.1 Remove the package from -20°C storage and allow the unopened package to thaw at 4°C for 6 to 24 hours on a flat surface before opening the package. **Do not stack plates during thawing.**

NOTE: Perform steps 1.2 through 1.4, **30 minutes** prior to seeding.

1.2 Under laminar flow hood, remove plates from the package.

1.3 Label plates with a white marker.

1.4 Remove and discard the mat cover with either sterile blunt-ended or tissue forceps after removing the lid. Replace the lid on the plate.

1.5 Allow Corning Matrigel® Matrix to polymerize for **30 minutes** at 37°C and 5% CO_2 environment. The plate is now ready for use. Prolonged polymerization leads to extensive monolayer formation rather than tube formation.

NOTE: Avoid using incubators in which CO_2 injection port is located at the top and CO_2 blows directly onto plates. Incubators in which CO_2 injection port places at bottom horizontally work well for tube formation.

2.0 Endothelial Cell Tube formation assay

NOTE: Assay procedure should be performed under aseptic conditions.

2.1 Prepare the Corning® BioCoat™ Angiogenesis Plate as directed above.

2.2 Culture endothelial cells with desired endothelial cell medium to desired confluence. For HUVEC and HMEC-1, 70-80% confluence is recommended.

2.2 Prepare endothelial cell suspensions by trypsinizing the cell monolayers and resuspending the cells in culture medium with 5 -10% serum or with your desired angiogenesis promoters at **$4x10^5$ cells/ml** when using HUVEC, HMVEC and HMEC-1. Additional testing agents such as inhibitory agents can be included at this step as well.

2.3 Add 50 µl of the cell suspension (**$2x10^4$ cells** of HUVEC, HMVEC and HMEC-1) to each well. An electronic multi-channel pipette works well when handling several plates in one experiment.

2.5 Incubate the Angiogenesis assay plate for 16 to 18 hours at 37°C, 5% CO_2 atmosphere.

3.0 Measurement of tube formation - Labeling with Corning Calcein AM Fluorescent Dye

NOTE: For each complete plate, 6.25 ml of HBSS and one 50 µg vial of Calcein AM are required. The use of HBSS is recommended as using culture medium results in the autohydrolysis of the label giving unacceptably high backgrounds. This step may be performed under non-aseptic conditions.

3.1 Prepare Calcein AM solution at 8 µg/ml. For each plate, measure out 6.25 ml of HBSS and warm to 37°C. Add 20 µl of DMSO to each 50 µg vial of calcein AM. Add approximately 100 µl of HBSS to the vial. Transfer the vial contents to the bulk of the HBSS.

3.2 Following incubation, carefully remove medium from the plates. Be careful not to disturb tubes that may have formed in the Corning Matrigel® Matrix. This can be accomplished manually by gently decanting the medium followed by gently blotting on a stack of paper towels.

3.3 Wash the plate with HBSS by adding 100 µl of HBSS to each well. Remove HBSS as described in 3.2

3.4 Repeat the wash once.

3.3 Label cells by adding 50 µl /well of 8 µg/ml Calcein AM in HBSS and incubate plates for 30-40 minutes at 37°C, 5% CO_2.

3.6 Remove the labeling solution as in 3.2

3.7 Wash the plates twice as in 3.3.

3.8 The plate now is ready for image acquisition using an automated imager or for taking pictures using a fluorescent microscope.

NOTE: Once hydrolysis occurs, calcein AM leaks out of cells resulting in a higher background. Labeled plates can be stored at 4°C for 1-2 hours with minimum increase in background.

3.9 Process the acquired images with the desired hardware and software. We use Gen-1 Cell-based Screening System and MetaMorph® software (Universal Imaging Corporation™, http://www.moleculardevices.com) to automatically acquire images and measure tube length.

3.10 If an automated image acquisition instrument is not available, it is possible to use a fluorescent microscope that is capable of taking picture manually, and process images using either MetaMorph or another equivalent software.

NOTE: Various researchers have measured a number of parameters such as tube length, tube areas, branch points. In the Corning® BioCoat™ Angiogenesis System: Endothelial Cell Tube Formations, tube formation is measured using the MetaMorph Software system. Some other commonly used imagining software packages for measuring the extent of tube formation include Image-Pro® Plus (Media Cybernetics http://www. mediacy. com/) and NIH *Image* (http://rsb.info.nih.gov/nih-image/index.html)

STABILITY

The Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation is stable when stored at **-20°C**. **DO NOT STORE IN FROST-FREE OR -70°C FREEZER.**

CUSTOMER AND TECHNICAL SUPPORT

To place an order in the U.S., contact Customer Service at:
tel: 800.492.1110, fax: 978.442.2476; email: CLSCustServ@corning.com.

For technical assistance, contact Technical Support at:
tel: 800.492.1110, fax: 978.442.2476; email: CLSTechServ@corning.com.

Outside the U.S., contact your local distributor or visit www.corning.com/lifesciences to locate your nearest Corning office.

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