

Corning® BioCoat™ Angiogenesis System: Endothelial Cell Migration

Catalog Nos. 354143, 354144, 354147, 354148

Guidelines for Use

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TABLE OF CONTENTS

Intended Use	3
Materials Provided	3
Related Products	3
General Information	4
Procedure for Use	5
1.0 Equilibration of inserts to room temperature	5
2.0 Migration Studies: Post-Labeling with Corning® Calcein AM Fluorescent Dye	5
3.0 Measurement of Cell Migration: Post-Labeling with Corning Calcein AM Fluorescent Dye	6
4.0 Migration Studies: Pre-Labeling and Measurement with Corning DiIC ₁₂ (3) Fluorescent Dye	7
5.0 Data Reduction	7
General Guidelines for Automated Use	8
Stability and Quality Control	8
Customer and Technical Service	9

INTENDED USE

The Corning® BioCoat™ Angiogenesis System: Endothelial Cell Migration provides endothelial cells with conditions that allow assessment of their migratory property *in vitro*. By combining the fluorescence blocking capabilities of the Corning FluoroBlok™ membrane with the benefits of an extracellular matrix, the process of screening for endothelial cell migration has been optimized. It is composed of a Falcon® 24- or 96-Multiwell Insert Plate containing a fluorescence blocking, microporous (3.0 µm pore size) PET membrane that has been evenly coated with Human Fibronectin. The concentration of Human Fibronectin is optimized to provide a suitable protein structure for adherent cells to attach to the membrane, and migrate freely in response to an angiogenic stimulus in the lower chamber of the insert plate.

Quantitation of cell migration is achieved by pre- or post-labeling cells with a fluorescent dye then measuring the fluorescence of migrating cells in a fluorescent plate reader. Since the Corning FluoroBlok membrane effectively blocks the passage of light from 490-700 nm at >99% efficiency, fluorescence from labeled cells that have not migrated are blocked from detection. However, cells that have migrated to the underside of the Corning FluoroBlok membrane are no longer shielded from the light source and are easily detected with a fluorescent plate reader. The fluorescent signal from the migrated cells can be directly correlated to cell number.

MATERIALS PROVIDED

- Cat. No. 354143 Corning BioCoat Angiogenesis System: Endothelial Cell Migration composed of one Corning FluoroBlok 24-Multiwell Insert Plate (3 micron pore size) coated with Human Fibronectin, and one Falcon 24-well plate (non-TC treated) with lid.
- Cat. No. 354144 Corning BioCoat Angiogenesis System: Endothelial Cell Migration composed of five Corning FluoroBlok 24-Multiwell Insert Plates (3 micron pore size) coated with Human Fibronectin and five Falcon 24-well plates (non-TC treated) with lids.
- Cat. No. 354147 Corning BioCoat Angiogenesis System: Endothelial Cell Migration composed of one Corning FluoroBlok 96-Multiwell Insert Plate (3 micron pore size) coated with Human Fibronectin and one Falcon 96-well plate (non-TC treated) with lid.
- Cat. No. 354148 Corning BioCoat Angiogenesis System: Endothelial Cell Migration composed of five Corning FluoroBlok 96-Multiwell Insert Plates (3 micron pore size) coated with Human Fibronectin, and five Falcon 96-well plates (non-TC treated) with lids

RELATED PRODUCTS

- Endothelial cell culture medium without added serum.
- Fetal bovine serum or appropriate Growth Factor such as Corning VEGF (Cat. No. 354107) as chemoattractant.
- Hanks' Balanced Salt Solution (HBSS).
- Fluorophore having excitation and emission wavelengths between 490 – 700 nm, for example:
Corning Calcein AM Fluorescent Dye, Catalog No. 354216 or 354217, or
Corning DiIC₁₂(3) Fluorescent Dye, Catalog No. 354218.
- DMSO (for Calcein AM).
- Falcon® 24- or 96-well plates for post cell invasion labeling (Cat. No. 351147 or 353928).

NOTE: A standard Falcon 96-well plate CANNOT be used.

- Humidified tissue culture incubator, 37°C, 5% CO₂ atmosphere.
- Corning® HUVEC-2, Human Umbilical Vein Endothelial Cells (Cat. No. 354151).
- BSA, delipidized (Cat. No. 354331).
- Laminar flow tissue culture hood.
- Fluorescence microplate reader with bottom reading capabilities.

GENERAL INFORMATION

The human plasma used in the preparation of this product has been tested and found negative for HBsAG and HIV antibody. Nevertheless, this product should be handled using the same safety precautions as are used when handling potentially infectious material.

Store materials at -20°C in the original packaging.

Addition of cells

All procedures should be performed under aseptic conditions.

Labeling of cells

Fluorescent dyes derived from the fluorescein, rhodamine and cyanine families may be used to label cells. For a listing of compatible fluorophores, please visit our website. Due to the nature of cell labeling, some fluorophores may disrupt the fluidity of the cell membrane, thus affecting cell function and viability. Titration of fluorophores is recommended to determine optimal concentration and incubation time for each cell type used.

Measurement of cells

Only those labeled cells that have invaded the Corning Matrigel® Matrix and passed through the pores of the Corning FluoroBlok membrane will be detected.

A fluorescent plate reader that has bottom-reading capability must be used.

It is of utmost importance that the Insert Systems are read using the correct plate map. For information on loading plate maps, go to our website at www.corning.com/lifesciences to access Document No. CLS-DL-CC-074 (Technical Bulletin #436), or contact Technical Service at CLSTechServ@corning.com.

Proper plate orientation is with well A1 at the top left corner and the Falcon logo oriented to the right as the plate is inserted into the reader.

Appropriate excitation and emission filters for detection of fluorophores used in cell labeling must be employed, unless a monochromator-based plate reader (e.g., Tecan Safire™) is available.

Use of an inverted fluorescence microscope to verify your results is highly recommended.

The lamp energy or gain setting may need to be determined empirically, but a midrange energy or midpoint gain should be a sufficient starting point. A gain setting that is too high may also lead to

saturation of the detector with the most highly fluorescent samples, which may prevent the acquisition of meaningful results.

Autofluorescence background

The Corning® FluoroBlok™ membrane exhibits negligible autofluorescence across the visible spectrum (490-700 nm) as demonstrated by top-reading fluorescence data. However, there is a low level of fluorescence background in bottom reading mode due to autofluorescence of and/or reflection from the polystyrene well bottom of the base plate. Use of excessively high gain settings or failure to run the appropriate controls can often give the false impression that the Corning FluoroBlok membrane blocks light inefficiently or has high inherent autofluorescence.

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

PROCEDURE FOR USE

The Corning BioCoat™ Angiogenesis System: Endothelial Cell Migration is evaluated and optimized for endothelial cell migration using HUVECs (human umbilical vein endothelial cells). Quantitation of cell migration is achieved by either pre- or post-labeling with fluorophores such as Corning DiIC₁₂(3) or calcein AM, and measuring the fluorescence of migrated cells. In the following procedure, cell labeling and assay conditions for quantitation of cell migration have been optimized for post-labeling with calcein AM to maximize the fluorescent signal while minimizing cytotoxic effects. Results may vary depending upon the cells and dye used and the specific conditions used, *e.g.*, pre- or post-cell labeling, medium, dye concentration, incubation time, cell seeding density, and chemoattractant. Conditions should be optimized for your own system.

1.0 Equilibrate to Room Temperature

Remove the package from **-20°C** storage and allow it to come to room temperature before opening.

2.0 Post-Labeling with Corning Calcein AM Fluorescent Dye

In this procedure, cells are labeled for quantitation after they have migrated through the Corning FluoroBlok membrane. As a result, only end-point measurements of cell migration may be obtained. For kinetic (real-time) data, pre-labeling is recommended. See Section 4.

2.1 The migratory competence of Primary HUVECs varies depending upon the individual from whom they are derived and passage level. As a result it is recommended that HUVECs used in assays that depend upon their migratory ability be prescreened for this activity and used at a low passage number. We recommend passage 8 or lower.

2.2 Growth of HUVECs in serum containing medium or in standard defined growth media containing factors such as VEGF immediately prior to preparing the suspension for the assay may result in diminished and/or variable response to endothelial cell attractants.

We recommend "starving" the cells for 4 - 5 hours prior to setting up the assay. This is accomplished by removing the growth medium, washing the monolayer and adding the basal medium (without serum or growth supplements) supplemented with 0.1% BSA.

- 2.3 HUVEC cell suspensions may be prepared by standard trypsinization techniques, however, release of the cells from the growth surface by non-enzymatic means results in preservation of cell surface proteins that may result in better maintenance of migratory response. The use of non-enzymatic cell dissociation solutions such as Specialty Media Cat. No. S-014-B is recommended.

NOTE: Most endothelial cell media does not contain a sufficient concentration of serum to deactivate trypsin. **The use of a trypsin neutralizing solution is recommended.**

- 2.4 Cell seeding density is somewhat dependent upon experimental conditions such as cell source, medium, and chemoattractant. As a starting point we recommend preparing suspension at 4.0×10^5 cells/ml or 3.3×10^5 cells/ml for 24- and 96-well plates, respectively. The recommended seeding volumes are 250 μ l or 75 μ l for 24- and 96-well plates, respectively.
- 2.5 The recommended volume of chemoattractant solution to be added to the plate well is 750 μ l and 225 μ l for 24- and 96-well plates, respectively. The recommended sequence of addition is: add cell suspension to the inserts, then add the chemoattractant solution to the plate well.

NOTE: To reduce air bubbles and optimize performance on fluorescence plate readers, fill the basal compartment using the sampling ports.

- 2.6 Incubate the Insert Plate for 22 ± 1 hour at 37°C, 5% CO₂.

3.0 Measurement of Cell Migration: Post-Labeling with Corning® Calcein AM Fluorescent Dye

NOTE: Calcein AM is used at 4 - 5 μ g/ml in HBSS. Using culture medium for the dilution of the fluorescent dye results in hydrolysis of the label giving higher backgrounds.

For each complete 24-Multiwell plate, one 50 μ g vial of calcein AM in 12.5 ml of HBSS is required. Each complete 96-Multiwell plate requires two 50 μ g vials of calcein AM in 25 ml of HBSS.

- 3.1 Add 20 μ l of DMSO to each 50 μ g vial of calcein AM. Add approximately 150 μ l of warm (37°C) HBSS to the vial. Transfer the vial contents to the bulk of the HBSS. Add approximately 150 μ l of warm HBSS to the vial again to rinse and give quantitative transfer of calcein AM.
- 3.2 Labeling can be easily done by either of two methods.
- The first method is adaptable to automated fluid handling and is useful when a large number of plates are being processed. The medium inside the insert and in the plate well is aspirated using the sampling port to gain access to the plate well. Wash the well and the underside of the membrane by adding HBSS (500 μ l for the 24-well plate; 200 μ l for the 96-well plate) to the plate well through the access port and again aspirating. Add 500 μ l of the calcein AM solution to the 24-well plate well or 225 μ l to the 96-well plate.
 - The second method is especially convenient when hand processing a smaller number of plates. The medium is removed by grasping the insert plate and flicking out the contents and material hanging from the underside. After removing the medium, the insert is transferred to a second plate containing the calcein AM solution (500 μ l per well for the 24-well plate or 225 μ l per well for the 96-well plate).

- 3.3 Incubate plates for 90 minutes at 37°C, 5% CO₂.
- 3.4 Fluorescence of invaded cells is read in a fluorescence plate reader with bottom reading capabilities at excitation/emission wavelengths of 494/517 nm. Only those labeled cells that have migrated through the pores of the Corning® FluoroBlok™ membrane will be detected.
- 4.0 Migration Studies: Pre-Labeling and Measurement with Corning DiIC₁₂(3) Fluorescent Dye (24-Multiwell Insert Format Only)

NOTE: Pre-labeling cells before they are added to the inserts allows you to run homogeneous, non-destructive assays in real-time. Kinetic data can be generated without dismantling or destroying the insert for each time point.

- 4.1 Equilibrate the Insert System as directed above.
- 4.2 Grow HUVECs to near confluence. Label cell monolayers *in situ* with desired fluorophore at desired concentration. Due to the nature of cell labeling, some fluorophores may disrupt or disturb the fluidity of the cell membrane, thus affecting cell function and viability. Titration of fluorophores is specifically recommended to determine optimal dye concentration and incubation time for each cell type used.
- For example, if you currently label cells at 10 µg/ml of DiIC₁₂(3) in medium containing 10% FBS for 1 hour at 37°C, label and incubate at various dye concentrations between 5 µg/ml and 10µg/ml and between 30 minutes to 1 hour to determine the optimal labeling conditions.
- 4.3 Refer to Section 2 of this document for volumes, cell seeding densities and helpful hints.
- 4.4 Take readings as desired. Fluorescence of invaded cells is read at wavelengths of 549/565 nm (Ex/Em). Only those labeled cells that have migrated and passed through the pores of the Corning FluoroBlok membrane will be detected.

5.0 Data Reduction

NOTE: The data may be expressed as either relative fluorescence units (RFU) or as “Fold Migration”, *i.e.*, the RFU value of the cells migrating through the fibronectin coated insert membrane in response to a chemoattractant (VEGF, FBS, or growth factors) relative to the RFU value of a control (no chemoattractant). Expressing data as “Fold Migration” is useful in normalizing data variability from different experiments due to differences in cell seeding number, viability, etc.

Correct for Background: Determine the mean value for the background insert wells and subtract it from each remaining value.

Data is expressed as the fold cell migration derived from the mean RFU of cell migration through the Corning FluoroBlok membrane towards a chemoattractant divided by the mean RFU of cell migration in the absence of chemoattractant.

$$\text{Fold Migration} = \frac{\text{Mean RFU of inserts with cells migrating through membrane towards chemoattractant}}{\text{Mean RFU of cells migrating through membrane without chemoattractant}}$$

GENERAL GUIDELINES FOR AUTOMATED USE

Handle all inserts under aseptic conditions.

If you plan to use this product with a robotic fluid handler, please note the following:

1. Corning® FluoroBlok™ 24- and 96-Multiwell Insert Systems are designed so that most robotic grippers can manipulate the entire assembly (insert, receiver tray and lid) as well as each individual component. The lid can be removed using standard robotic grippers (i.e. Tecan RoMa, Thermo-CRS Catalyst Express). The lid can also be removed from above using a suction or vacuum based delidding station (i.e. Thermo-CRS). To avoid splashing the media, with some robotic delidding stations, we recommend programming the automation to perform smooth, slow movements.
2. To prevent cross contamination of wells, the insert plate is designed to be placed in a Falcon® 24-well plate (Cat. Nos. 351147 and 353047) or 96-Square Well Flat Bottom Plate (Cat. No. 353928) in one unique orientation. To properly align the insert plate in the 24- or 96-well plate, make sure the Falcon logo, on the top of both pieces, faces the same direction.
3. For the 96-Multiwell Insert Plate, fixed tips or disposable tips with narrow or tapered profiles should be used. Tips that have a large outer diameter may stick in the access ports. Most automation vendors supply compatible tips.
4. For the 24-Multiwell Insert plate, any size disposable pipet tip may be used to access the basal chamber. These include 50, 100, 200, 250, and 1000 ul formats.

STABILITY AND QUALITY CONTROL

The Corning BioCoat™ Angiogenesis System: Endothelial Cell Migration is stable when stored at -20°C.

- Each lot is tested for its ability to support migration of HUVEC (human umbilical vein endothelial cells) in response to VEGF (vascular endothelial cell growth factor).
- Each lot is tested and found negative for bacteria and fungi.

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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