

BD BioCoat™ Angiogenesis System: Endothelial Cell Invasion

Catalog Nos. 354141 and 354142

Guidelines for Use

**FOR RESEARCH USE ONLY.
NOT FOR CLINICAL, DIAGNOSTIC, or THERAPEUTIC PROCEDURES.
NOT FOR USE IN HUMANS.**



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INTENDED USE

BD BioCoat™ Angiogenesis System: Endothelial Cell Invasion provides endothelial cells with conditions that allow assessment of their invasive property *in vitro*. It consists of a BD Falcon™ FluoroBlok™ 24-Multiwell Insert Plate with a 3.0 micron pore size Polyethylene Terephthalate (PET) membrane that has been uniformly coated with BD Matrigel™ Matrix. The uniform layer of Matrigel Matrix provides an authentic barrier to non-invasive cells. The BD Matrigel Matrix coating process occludes the pores of the membrane, blocking non-invasive cells from migrating through the membrane. By contrast, endothelial cells (malignant and non-malignant cells) are able to detach themselves from and migrate through the BD Matrigel Matrix-treated membrane.

Quantitation of cell invasion is achieved by pre- or post-labeling cells with a fluorescent dye and measuring the fluorescence of invading cells in a fluorescent plate reader. Since the BD FluoroBlok membrane effectively blocks the passage of light from 490-700 nm at >99% efficiency, fluorescence from labeled cells that have not invaded the BD Matrigel Matrix and passed through the BD FluoroBlok membrane is blocked from detection. Cells that have invaded to the underside of the BD FluoroBlok membrane are no longer shielded from the light source and are easily detected with a fluorescent plate reader. Alternatively, an inverted fluorescence microscope may be used to view each well. If the microscope is equipped with a camera, photographs may be taken of each well and processed with image analysis software such as NIH *Image*, which is available free of charge from the NIH.

MATERIALS PROVIDED

Cat. No. 354141, BD BioCoat Angiogenesis System: Endothelial Cell Invasion, one per pack.

Cat. No. 354142, BD BioCoat Angiogenesis System: Endothelial Cell Invasion, five per pack.

RELATED PRODUCTS

Endothelial cell culture medium without added serum

Chemoattractant such as 5% Fetal Bovine Serum (FBS) in tissue culture medium or appropriate Growth Factor such as VEGF (BD Cat. No. 354107)

Delipidized BSA (BD Cat. No. 354331)

Hanks' Balanced Salt Solution (HBSS)

Fluorophore having excitation and emission wavelengths between 490-700 nm, e.g., BD Calcein AM Fluorescent Dye (Cat. No. 354216 or 354217) or BD DiIC₁₂(3) Fluorescent Dye (Cat. No. 354218)

Dimethyl Sulfoxide (DMSO) for dissolving calcein AM

BD Falcon 24-well plates for post cell invasion labeling (Cat. No. 351147)

BD Falcon™ FluoroBlok™ 24-Multiwell Insert System (**uncoated**) (Cat. No. 351155, 1-plate pack or Cat. No. 351156, 5-plate pack) to be used as a cell migration control

Cells such as Human Microvascular Endothelial Cells, HMEC-1 (CDC/Emory University) or NIH 3T3 cells (ATCC).

Fluorescence Plate Reader with bottom-reading capabilities (i.e., PerkinElmer Victor™, Tecan GENios™, and others)

PRECAUTIONS

Storage: Materials should be stored at -20°C in the original packaging.

All procedures should be performed under aseptic conditions.

California Proposition 65 Notice

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

GENERAL USE

NOTE: Fluorescent dyes derived from the fluorescein, rhodamine, and cyanine families may all be used to label cells. For a listing of compatible fluorophores, visit our website at www.bdbiosciences.com/discovery_labware. Due to the nature of cell labeling, 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.

Procedure:

To calculate percent invasion, a cell migration control plate (**uncoated insert, not included**) is required.

1.0 Rehydration

- 1.1 Remove the package from **-20°C** storage and allow package to come to room temperature before opening the package.
- 1.2 Add 0.5 ml warm (37°C) basal culture media to the interior of the insert wells. Allow to rehydrate for **15-45 minutes** at 37°C in a CO₂ environment. The plate is now ready to use.
- 1.3 After rehydration, carefully remove the medium from the insert wells without disturbing the layer of BD Matrigel™ Matrix on the membrane. This can be easily accomplished by careful aspiration or by inverting the plate and “flicking” the solution from the entire plate at once. **It is important not to disturb the layer of Matrigel Matrix during this process.**

2.0 Invasion Studies: Post-Labeling with BD Calcein AM Fluorescent Dye

The BD BioCoat™ Angiogenesis System: Endothelial Cell Invasion is evaluated and optimized for invasivity using HMEC-1 cells (CDC/Emory University), a human microvascular endothelial cell line, and NIH 3T3 cells, a mouse fibroblast cell line of low invasivity. Quantitation of cell invasion is achieved by either pre- or post-cell invasion labeling with fluorophores such as DiIC₁₂(3) or

Calcein AM, respectively, and measuring the fluorescence of invading cells. [Cell labeling and assay conditions have been optimized to maximize the fluorescent signal while minimizing the cytotoxic effects of Calcein AM on HMEC-1 and NIH 3T3 cells]. Results may vary depending upon the cells and dye used and the specific conditions (i.e., pre- or post-cell labeling) especially those of medium, dye concentration, incubation time, cell seeding density, and chemoattractant, under which the procedure is performed. Individual researchers should optimize conditions for their system.

NOTE: In this procedure, cells are labeled for quantitation after they have invaded the BD Matrigel™ Matrix and passed through the BD FluoroBlok™ membrane. As a result, **only end-point measurements of cell invasion** may be obtained using post-cell invasion labeling.

- 2.1 Prepare and rehydrate the Insert Plate as directed above. It is not necessary to rehydrate the **(uncoated)** BD Falcon™ FluoroBlok 24-Multiwell Insert Plate that will be used as a cell migration control.
 - 2.2 Prepare cell suspensions by trypsinizing the cell monolayers and resuspending the cells in culture medium without serum at **2.0 x 10⁵ cells/ml**. To determine the optimal seeding density for your cell type on a porous growth surface, we recommend using a range of seeding densities (cells/cm²) that bracket the seeding density used on nonporous surfaces (i.e., flasks, dishes, and plates). For example, if you currently seed at 10⁵ cells/cm², seed at various cell concentration between 0.5 x 10⁵ and 5.0 x 10⁵ cells/cm² to determine the optimal initial seeding density.
 - 2.3 Add 0.25 ml of cell suspension (**5.0 x 10⁴ cells/top chamber**) to the top chambers.
 - 2.4 Using the sample ports, immediately add 750 µl of culture medium containing 5% FBS or appropriate growth factor, such as BD™ VEGF (at appropriate concentration) as a chemoattractant to each of the bottom wells.
 - 2.5 Incubate the Insert Plate and the uncoated Control Plate for 22 ± 1 hour at 37°C, 5% CO₂ atmosphere.
- 3.0 Measurement of Cell Invasion: Post-Labeling with BD Calcein AM Fluorescent Dye

NOTE: For each complete plate, 12.5 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 fluorescence label giving an unacceptably high background.

- 3.1 Prepare calcein AM solution at 4 µg/ml. For each plate, measure out 12.5 ml of HBSS and warm to 37°C. Add 20 µl of DMSO to each 50 µg vial of calcein AM. Add approximately 150 µl of warm (37°C) HBSS to the vial. Transfer the vial contents to the bulk of the HBSS. Re-add approximately 150 µl of warm HBSS to the vial again to rinse and give quantitative transfer of calcein AM.
- 3.2 Following incubation, carefully remove medium from the upper chambers. Be careful not to transfer any drops of medium that may be hanging from the insert bottom to adjacent wells. This can be easily accomplished by grasping the insert plate and “flicking” out the contents and the material hanging from the underside. **It is important not to disturb the layer of BD Matrigel Matrix during this process.**
- 3.3 Transfer the insert plate into a second BD Falcon 24-well plate containing 0.5 ml/well of 4 µg/ml calcein AM in HBSS and 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 invaded the BD Matrigel™ Matrix and passed through the pores of the BD FluoroBlok™ membrane will be detected.

NOTE: Other fluorescent dyes can be used for post-labeling that include, but are not limited to nuclear stains such as SYTO-24. Unlike calcein AM, SYTO-24 has low background in serum containing solutions, and therefore may not require a second 24-well plate for labeling.

4.0 Invasion Studies: Pre-Labeling with BD DiIC₁₂(3) Fluorescent Dye

NOTE: Pre-labeling cells before they are added to the inserts allows homogeneous assays to be run. Real time kinetic data can be generated to chart cell invasion through the BD Matrigel Matrix and through the BD FluoroBlok membrane without dismantling or destroying the insert for each time point.

- 4.1 Rehydrate the Insert Plate as directed in Section 1.0. It is not necessary to rehydrate the uncoated BD Falcon™ FluoroBlok 24-Multiwell Insert Plate that will be used as a cell migration control.
- 4.2 Grow HMEC-1 and NIH 3T3 cells 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. Results may vary depending upon the cells and dye used and the specific conditions, especially those of medium, dye concentration, incubation time, cell seeding density, and chemoattractant, under which the procedure is performed. Individual researchers should optimize conditions for their system. 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 10 µg/ml and between 30 minutes to 1 hour to determine the optimal labeling conditions.
- 4.3 Prepare cell suspensions by trypsinizing the cell monolayers and resuspending the cells in appropriate culture medium without serum at **2 x 10⁵ cells/ml**. To determine the optimal seeding density for your cell type on a porous growth surface, we recommend using a range of seeding densities (cells/cm²) that brackets the seeding density used on nonporous surfaces (i.e., flasks, dishes, and plates). For example, if you currently seed at 10⁵ cells/cm², seed at various cell concentrations between 0.5 x 10⁵ and 5 x 10⁵ cells/cm² to determine the optimal initial seeding density.
- 4.4 Add 0.25 ml of labeled cell suspension (**5 x 10⁴ cells/top chamber**) to the top chambers.
- 4.5 Using the sample ports, immediately add 750 µl of media containing 5% FBS or appropriate growth factor, such as BD™ VEGF (at appropriate concentration) as a chemoattractant to each of the bottom wells.
- 4.6 Incubate the Insert Plate and the uncoated Control Plate for 22 ± 1 hour at 37°C, 5% CO₂ atmosphere.

5.0 Measurement of Cell Invasion: Pre-Labeling with BD DiIC₁₂(3) Fluorescent Dye

Take readings as desired: fluorescence of invaded cells is read directly in a fluorescence plate reader with bottom reading capabilities at appropriate excitation/emission wavelengths for the fluorophore used (549/565 nm for DiIC₁₂(3)). Only those labeled cells that have invaded the BD Matrigel™ Matrix and passed through the pores of the BD FluoroBlok membrane will be detected.

6.0 Data Reduction

NOTE: The data may be expressed as either relative fluorescence units (RFU) or as “Percent Invasion”, *i.e.*, the RFU value of the cells invading through the BD Matrigel™ Matrix coated insert membrane relative to the RFU value of the cells migrating through an uncoated insert membrane. Expressing data, as “Percent Invasion” is useful in normalizing data variability from different experiments due to differences in cell seed, viability, etc.

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

- 6.1 Data is expressed as the mean percent cell invasion derived from the mean RFU of cell invasion through the **BD Matrigel Matrix-coated** BD FluoroBlok™ inserts divided by the mean FU of cell migration through the **uncoated** BD FluoroBlok control inserts × 100.

Determine the Percent Cell Invasion:

$$\% \text{ Invasion} = \frac{\text{Mean RFU of cells invading through BD Matrigel Matrix-coated BD FluoroBlok membrane}}{\text{Mean RFU of cells migrating through uncoated BD FluoroBlok control insert}} \times 100$$

- 6.2 Data may also be expressed as the Signal to Noise (S/N) ratio derived from the ratio of the RFU of endothelial cell invasion (Test cells) over the RFU of NIH 3T3 cell invasion (Control cells).

Determine the Signal to Noise ratio:

$$S/N = \frac{\text{RFU of Test cell invasion (i.e., HMEC-1 cell invasion)}}{\text{RFU of Control cell invasion (i.e., NIH 3T3 cell invasion)}}$$

GUIDELINES FOR AUTOMATED USE

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

1. BD Falcon™ FluoroBlok™ 24-Multiwell Insert Systems are designed so that 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 may also be removed from above using suction or a vacuum-based delidding station (i.e., Thermo-CRS). To avoid splashing the media 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 BD Falcon™ 24-well plate (Cat. Nos. 351147 or 353047) in one unique orientation. To properly align the insert plate within the BD Falcon 24-well plate, make sure the Falcon logo, on the top of both pieces, face the same direction.
3. Any size disposable pipet tip may be used to access the basolateral chamber. These include 50, 100, 200, 250, and 1000 µl formats.

GENERAL PLATE READER SETUP DETAILS FOR 24-MULTIWELL INSERT SYSTEMS

An important aspect of obtaining optimal performance with the BD Falcon™ and BioCoat™ FluoroBlok™ Insert Products is the selection of an appropriate fluorescence plate reader. As most cell or fluorophore migration/invasion experiments are monitored by the appearance of fluorescence in the lower chamber, bottom-reading capability is required for use with BD FluoroBlok Insert Products.

It is critical that the Insert Systems are read using the proper template, as in most cases the standard 24-well plate map, which may be available from the software pull down menu, does not read the entire bottom of the well which leads to erroneous results. For more information on loading the proper template, see BD Biosciences Discovery Labware Technical Bulletin #436 located on our website at www.bdbiosciences.com/discovery_labware or call Technical Service at 800.343.2035 (978.901.7300 outside of North America).

For plate readers with a center-read on/off option, select the off position and enable the 4 reads per well setting.

INSERT SYSTEM ASSEMBLY & ORIENTATION

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

AUTOFLUORESCENCE BACKGROUND

The BD FluoroBlok™ PET membrane exhibits negligible autofluorescence across the visible spectrum (490-700 nm), as is evident by top-reading fluorescence data. However, there is a slight but noticeable fluorescence background in bottom reading due to autofluorescence of and/or reflection from the polystyrene well bottom of the base 24-well plate. Use of excessively high gain (lamp energy, etc) in the plate reader settings, or failure to run the appropriate background or negative controls, can often give the false impression that the BD FluoroBlok membrane blocks light inefficiently or has high inherent autofluorescence. A gain

setting that is too high may also lead to saturation of the detector with the most highly fluorescent samples, which may prevent meaningful results from being acquired. The exact settings may need to be determined empirically, however a gain setting of 50% of max , or a midrange lamp energy is a good starting point.

FLUORESCENCE DETECTION ISSUES

Appropriate excitation and emission filters for detection of fluorophore(s) used in cell labeling must be employed, unless a monochromatic-based plate reader (i.e., Tecan Safire™) is available. To ensure that all samples are measured as accurately as possible, an appropriate gain setting or lamp intensity setting must be used (see Autofluorescence Background).

CUSTOMER AND TECHNICAL SERVICE

For technical assistance, contact Technical Service at:
Tel: 877.232.8995 or 978.901.7389 Fax: 978.901.7491; e-mail: Labware@bd.com

To place an order in the U.S., contact Customer Service at;
Tel: 877.232.8995 Fax: 800.325.9637 or 858.812.8889

Outside the U.S., contact your local distributor or nearest BD Biosciences office.

Visit our website www.bdbiosciences.com/discovery_labware for additional information on BD BioCoat or products including:

- Product Literature
- Bibliography
- List of Related Products

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