

BD BioCoat™ Tumor Invasion System

96-Multiwell Format

Catalog Nos. 354167 and 354168

Guidelines for Use

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



BD Biosciences – Discovery Labware

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INTENDED USE

The BD BioCoat™ Tumor Invasion System provides cells with conditions that allow assessment of their invasive potential *in vitro*. It consists of a BD Falcon™ FluoroBlok™ 96-Multiwell Insert Plate with an 8.0 micron pore size PET membrane that has been uniformly coated with BD Matrigel™ Matrix. This uniform layer of BD Matrigel Matrix serves as a reconstituted basement membrane *in vitro* providing a true barrier to non-invasive cells while presenting an appropriate protein structure to study invasion. The coating process occludes the pores of the membrane, blocking non-invasive cells from migrating through the membrane. In contrast, invasive cells (malignant and non-malignant cells) are able to detach themselves from and migrate through the coated membrane.

Quantitation of cell invasion is achieved by either pre- or post-cell invasion labeling with a fluorescent dye such as DiIC₁₂(3) or calcein AM, respectively, and measuring the fluorescence of invading cells. Since the BD FluoroBlok membrane effectively blocks the passage of light from 490-700 nm at >99% efficiency, fluorescently-labeled cells that have not invaded are not detected by a bottom-reading fluorescence plate reader. However, cells that have invaded to the underside of the membrane are no longer shielded from the light source and are detected with the respective plate reader.

MATERIALS PROVIDED

- Catalog No. 354167, BD BioCoat Tumor Invasion System, composed of a BD Falcon FluoroBlok 96-Multiwell Insert Plate (8 micron pore size) coated with BD Matrigel Matrix, a BD Falcon 96-well plate (non-TC treated) and lid.
- Catalog No. 354168, BioCoat Tumor Invasion System, composed of five BD Falcon FluoroBlok 96-Multiwell Insert Plates (8 micron pore size) coated with BD Matrigel Matrix, five 96-well plates (non-TC treated) and lids.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Cells such as HT-1080 or NIH/3T3 (ATCC)
- Bicarbonate based culture medium, e.g., Dulbecco's Modified Eagle Medium (DMEM; serum-free)
- Dulbecco's Phosphate Buffered Saline (DPBS)
- BD Falcon FluoroBlok™ 96-Multiwell Insert System to be used as a cell migration control, Catalog No. 351163, 1-plate pack or Catalog No. 351164, 5-plate pack
- Chemoattractant such as 5% fetal bovine serum in DMEM
- Fluorophore having excitation and emission wavelengths between 490 – 700 nm, for example: BD Calcein AM Fluorescent Dye, Catalog No. 354216 or 354217, or BD DiIC₁₂(3) Fluorescent Dye, Catalog No. 354218
- Hanks' Balanced Salt Solution (HBSS)
- BD Falcon™ 96-square well plates for post cell invasion labeling, Catalog No. 353928

- Fluorescence plate reader with bottom reading capabilities (e.g., PerkinElmer Victor™, TECAN GENios Pro™, Molecular Devices SpectraMax® M5, BioTek Synergy™, BMG FLUOstar OPTIMA)

QUALITY CONTROL

- Each lot of BD BioCoat™ Tumor Invasion System is tested for its ability to allow invasion of HT-1080 cells, a highly invasive human fibrosarcoma cell line, and to exclude NIH/3T3, a weakly invasive mouse fibroblast cell line.
- All lots are tested and found negative for bacteria and fungi.

GENERAL INFORMATION

Addition of cells

All procedures should be performed under aseptic conditions.

Grow cells to ~80% confluence.

It is not necessary to rehydrate the uncoated BD Falcon FluoroBlok™ 96-Multiwell Insert System that will be used as a cell migration control, but 25 µL of medium must be added prior to cell addition.

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 2.5 ×10⁵ cells/cm², seed at various cell concentrations between 5 ×10⁴ and 5 ×10⁵ cells/cm² to determine the optimal initial seeding density.

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 BD Matrigel™ Matrix and passed through the pores of the BD Falcon 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, see Technical Bulletin #436 on our website, or contact Technical Service.

Proper plate orientation is with well A1 at the top left corner and the BD 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.

Use of autogain (if supported on your reader) is not recommended.

Autofluorescence background

The BD Falcon™ 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 BD Falcon FluoroBlok membrane blocks light inefficiently or has high inherent autofluorescence.

PROCEDURE FOR USE

The following procedure is optimized for cell labeling and assay conditions that maximize the fluorescent signal while minimizing the cytotoxic effects of these fluorophores on HT-1080 and NIH/3T3 cells. Results may vary depending upon the cells and dye used and the specific conditions for the procedure (medium, dye concentration, incubation time, cell density, chemoattractant, etc.). Conditions should be optimized for your own system.

1.0 Rehydration

- 1.1 Remove the package from **-20°C** storage and allow it to come to room temperature.
- 1.2 Open the foil package and add 75 µL warm (37°C) DMEM to each apical chamber. Allow the plate to rehydrate for **2 hours** at 37°C in a 5% CO₂ environment.
- 1.3 After rehydration, carefully remove 50 µL of medium from each apical chamber without disturbing the layer of BD Matrigel™ Matrix on the membrane. The system is now ready to use.

2.0 Post-labeling and measurement using BD Calcein AM Fluorescent Dye (Cat nos. 354216 or 354217)

In this sub-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 measurement of cell invasion may be obtained. For kinetic (real-time) data, pre-labeling is recommended (See section 3).

- 2.1 Prepare and rehydrate the insert system as directed above.
- 2.2 Prepare cell suspensions by trypsinizing the cell monolayers and resuspending the cells in serum-free DMEM at **5 × 10⁵ cells/mL**.
- 2.3 Add 25 μL of cell suspension (1.25 × 10⁴ cells) to the apical chambers. Add 25 μL of serum-free medium to each apical chamber in the uncoated system.
- 2.4 Using the sample ports for access, add 200 μL of chemoattractant to each basal chamber.
- 2.5 Incubate the BD BioCoat™ Tumor Invasion System and the uncoated BD Falcon FluoroBlok™ 96-Multiwell Insert System for 20 – 22 hours (dependent on cell type) at 37°C, 5% CO₂.
- 2.6 Following incubation, carefully remove medium from the apical chambers. This can be accomplished by flicking the contents into a waste container. Do not touch the bottom surface of the insert system.
- 2.7 Transfer the insert system into a second 96-square well plate containing 200 μL/well of 4 μg/mL calcein AM in HBSS. Incubate for 1 hour at 37°C, 5% CO₂.
- 2.8 Fluorescence of invaded cells is read at wavelengths of 494/517 nm (Ex/Em).

3.0 Pre-labeling and measurement using BD DiIC₁₂(3) Fluorescent Dye (Cat no. 354218)

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 to chart cell invasion without dismantling or destroying the insert for each time point.

- 3.1 Prepare and rehydrate the insert system as directed above.
- 3.2 Label cell monolayers *in situ* with 10 μg/ml of DiIC₁₂(3) in DMEM containing 10% FBS for 1 hour at 37°C.
- 3.3 Prepare cell suspensions by trypsinizing the cell monolayers and resuspending the cells in serum-free DMEM at **5 × 10⁵ cells/mL**.
- 3.4 Add 25 μL of the labeled cell suspension (1.25 × 10⁴ cells) to the apical chambers. Add 25 μL of serum-free medium to each apical chamber in the uncoated system.
- 3.5 Using the sample ports for access, add 200 μL of chemoattractant to each basal chamber.

- 3.6 Incubate the BD BioCoat™ Tumor Invasion System and the uncoated BD Falcon FluoroBlok™ 96-Multiwell Insert for 20 – 22 hours (dependent on cell type) at 37°C, 5% CO₂ atmosphere. Take readings as desired. Fluorescence of invaded cells is read at wavelengths of 549/565 nm (Ex/Em).

4.0 Data reduction

Data is expressed as the in the following equation :

$$\% \text{ invasion} = \frac{\text{Mean RFU of cells invaded through BD Matrigel™ Matrix coated membrane towards chemoattractant}}{\text{Mean RFU of cells migrated through uncoated BD FluoroBlok membrane towards chemoattractant}} \times 100$$

Background may be subtracted prior to the calculation of percent cell invasion.

RFU = relative fluorescent units.

GENERAL GUIDELINES FOR AUTOMATED USE

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

1. BD Falcon FluoroBlok™ 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 (e.g. TECAN RoMa, Thermo Electron CRS CataLyst Express). The lid can also be removed from above using suction or vacuum based delidding station (*i.e.*, Thermo-CRS). To minimize splashing of medium, we recommend programming the automation system to perform smooth, slow movements.
2. To prevent cross-contamination of wells, the insert plate is designed to be placed in a BD Falcon™ 96-Square Well Flat Bottom Plate (Cat. No. 353928) in one unique orientation. To properly align the insert plate in the square well plate, make sure the BD Falcon logos on the top of both parts are oriented in the same direction.
3. For the 96-Multiwell Insert System, 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. Contact technical service for specific information.

STORAGE AND STABILITY

When stored at –20°C in the original packaging, the BD BioCoat™ Tumor Invasion System is stable for at least 3 months from date of shipment.

California Proposition 65 Notice

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

Component: Chloroform

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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