

BD BioCoat™ Matrigel™ Invasion Chamber

Catalog No. 354480, 354481

Guidelines for Use

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

BD Biosciences

Clontech
Discovery Labware
Immunocytometry Systems
Pharming

BD Biosciences Discovery Labware

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INTENDED USE

The BD BioCoat™ Matrigel™ Invasion Chamber is useful to study cell invasion of malignant and normal cells. Specific applications include assessment of the metastatic potential of tumor cells¹, inhibition of metastasis by extracellular matrix components² or antineoplastic drugs (taxol)³, altered expression of cell surface proteins⁴ or metalloproteinases⁵ in metastatic cells, and invasion of normal cells, such as embryonic stem cells⁶, cytotrophoblasts⁷, and fibroblasts⁸.

Invasion studies have been successfully performed on a variety of tumor cells (cell lines and primary tumors) including melanomas, glioblastomas, astrocytomas, osteosarcomas, fibrosarcomas, and adenocarcinomas of the lung, prostate, breast, ovary, and kidney.

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SUMMARY

BD BioCoat Matrigel Invasion Chambers provide cells with the conditions that allow assessment of their invasive property *in vitro*. The **BD BioCoat Matrigel Invasion Chambers** consists of a **BD Falcon™ TC Companion Plate** with **Falcon Cell Culture Inserts** containing an 8 micron pore size PET membrane with a thin layer of **MATRIGEL** Basement Membrane Matrix. The Matrigel Matrix serves as a reconstituted basement membrane *in vitro*. The layer occludes the pores of the membrane, blocking non-invasive cells from migrating through the membrane. In contrast, invasive cells (malignant and non-malignant) are able to detach themselves from and invade through the Matrigel Matrix and the 8 micron membrane pores. The membrane may be processed for light and electron microscopy and can be easily removed after staining. The **MATRIGEL** Invasion Chamber is a convenient, ready-to-use system to study cell invasion *in vitro*.

MATERIALS PROVIDED

- Invasion Chambers (24 inserts each)
- Catalog # 354480 provided as 12 inserts in each of two 24-well BD Falcon TC Companion Tissue Culture Plates
- Catalog # 354481 provided as 6 inserts in each of four 6-well BD Falcon TC Companion Tissue Culture Plates

MATERIALS REQUIRED BUT NOT SUPPLIED

- Bicarbonate based culture medium such as **DMEM (serum-free)**
- Control Inserts (24-well, Catalog #354578 or 6-well, Catalog #354576)
- Chemoattractant such as 5% fetal bovine serum in tissue culture medium
- Companion Plates (24-well, Catalog #353504 or 6-well, Catalog #353502)
- Humidified tissue culture incubator, **37° C, 5% CO₂** atmosphere

- Diff-Quik staining kit (Allegiance Catalog # B4132-1A) or other suitable fixative and stain
- Laminar flow tissue culture hood
- Scalpel (#11 blade recommended)
- Microscope with (optional) camera
- Microscope slides and coverslips
- Cotton swab
- Sterile forceps
- Immersion oil

PRECAUTIONS

- a.) The following procedure has been optimized using HT-1080 human fibrosarcoma cells. Results may vary depending upon the cells used and the specific conditions, especially those of medium, incubation time, cell seeding density, and chemoattractant, under which the procedure is performed. Individual researchers should optimize conditions for their system.
- b.) **Storage: Materials should be stored at -20°C in the original packaging.**
- c.) **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

PROCEDURE FOR USE

NOTE: The appearance of the Matrigel Matrix coating on the BD BioCoat Matrigel Invasion Chamber has changed. The new glossy appearance is a result of process improvements, which have enhanced the performance and quality of the product.

1.0 Rehydration

Note: Refer to **Table 1** below for volumes to be used for the 24-well (354480) or 6-well (354481) configurations of the BD BioCoat™ Matrigel™ Invasion Chambers.

- 1.1 Remove the package from -20°C storage and allow to come to room temperature.
- 1.2 Add warm (37°C) bicarbonate based culture medium to the interior of the inserts

and bottom of wells. Allow to rehydrate for **2 hours** in humidified tissue culture incubator, 37°C, 5% CO₂ atmosphere.

- 1.3 After rehydration, carefully remove the medium without disturbing the layer of Matrigel™ Matrix on the membrane.

Table 1: Solution Volumes for Matrigel Invasion Chambers		
	24-well	6-well
Rehydration of insert and well	0.5 ml (insert) and 0.5 ml (well)	2.0 ml (insert) and 2.0 ml (well)
Well (i.e., chemoattractant)	0.750 ml	2.5 ml
Cells	0.50 ml	2.0 ml
Stain	0.50 ml	2.5 ml
Rinse	150 ml	250 ml

2.0 Invasion Studies

- 2.1 Rehydrate the number of Matrigel inserts to be used as directed above. Prepare an equal number of Control Inserts by using sterile forceps to transfer them to empty wells of the BD Falcon™ TC Companion Plate.
- 2.2 Prepare HT-1080 cell suspensions in culture medium containing **5x10⁴ cells/ml** for 24-well chambers or **1.25x10⁵ cells/ml** for 6-well chambers. 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 0.5x10⁵ and 5x10⁵ cells/cm² to determine the optimal initial seeding density.
- 2.3 Add chemoattractant to the wells of the BD Falcon TC Companion Plate.
- 2.4 Use sterile forceps to transfer the chambers and control inserts to the wells containing the chemoattractant. Be sure that no air bubbles are trapped beneath the membranes. This can be avoided by tipping the insert or chamber at a slight angle as it is lowered into the liquid.
- 2.5 Immediately add 0.5 ml of HT-1080 cell suspension (**2.5x10⁴ cells**) or your cell suspension to the 24-well chambers or 2.0 ml (**2.5x10⁵ cells**) to the 6-well chambers.
- 2.6 Incubate the BD BioCoat Matrigel Invasion Chambers for **22** hours in a humidified tissue culture incubator, at 37°C, 5% CO₂ atmosphere.

3.0 Measurement of cell invasion

- 3.1 Removal of non-invading cells

Note: After incubation, the non-invading cells are removed from the upper surface of the membrane by "scrubbing". The attachment of the membrane to the insert housing is quite firm and will not be dislodged during scrubbing nor will cells be dislodged from the bottom surface of the membrane. Scrubbing is very efficient in removing Matrigel Matrix and/or non-invading cells from the upper membrane surface. **Scrubbing must be accomplished quickly to avoid drying of the cells adhering to the bottom surface of the membrane.**

- a.) Insert a cotton tipped swab into the BD BioCoat™ Matrigel™ insert and apply gentle but firm pressure while moving the tip over the membrane surface.
- b.) Repeat the scrubbing with a second swab moistened with medium.

3.2 Staining of cells

Note: The cells on the lower surface of the membrane are stained with Diff-Quik™ stain. The Diff-Quik kit contains a fixative and two stain solutions. Staining is accomplished by sequentially transferring the inserts through the three solutions and two water rinses. The appearance is similar to that obtained by Wright-Giemsa staining. The cell nuclei stain purple and the cytoplasm stains pink. Suitable alternative staining procedures include fixation followed by hematoxylin and eosin staining or crystal violet. The membranes need not be removed from the insert housing for staining.

- a.) Add each Diff-Quik solution to three rows of a BD Falcon™ TC Companion Plate. Add distilled water to two beakers.
- b.) Sequentially transfer the inserts through each stain solution and the two beakers of water. Allow approximately > 2 minutes in each solution.
- c.) Allow the inserts to air dry.

Alternatively, cells may be fixed and stained with 100% methanol and 1% Toluidine blue, respectively.

- a.) Add 100% methanol to the appropriate number of wells of a BD Falcon™ TC Companion Plate. In a separate plate, add 1% Toluidine Blue in 1% borax to the appropriate number of wells. Add distilled water to two beakers.
- b.) Transfer inserts into the methanol for 2 minutes.
- c.) Transfer inserts into the Toluidine stain for 2 minutes.
- d.) Rinse inserts in the two beakers of distilled water to remove excess stain.
- e.) Allow the inserts to air dry.

3.3 Counting of invading cells

Note: Cell counting is facilitated by photographing the membrane through the microscope. Direct counting of the cells at the microscope is also acceptable.

- a.) Remove the membrane from the insert housing by inverting the insert and inserting the tip of a sharp scalpel blade through the membrane at the edge adjacent to the housing wall. Rotate the insert housing against the stationary blade and the membrane will be released in much the same manner as the lid is cut from a tin can. Do not fully release the membrane from the housing but leave a very small point of attachment.
- b.) Use forceps to peel the membrane from the remaining point of attachment and place it bottom side down on a microscope slide on which a small drop of immersion oil has been placed. Place a second very small drop of immersion oil on top of the membrane.
- c.) Place a second slide or cover slip on top of the membrane and apply gentle pressure to expel any air bubbles.
- d.) Observe and/or photograph the invading cells under the microscope at approximately 40 - 200X magnifications depending on cell density. Count cells in several fields of triplicate membranes.

Note: Cells will invade through the Matrigel™ on the 8 μm membrane pores ranging from even distribution to localization in discrete areas notably the center of the membrane and/or around the periphery of the membrane. When counting cells of triplicate membranes, choose fields in the center of the membrane as well as fields in the periphery of the membrane for 'true' representation of the cell number throughout the membrane.

3.4 Data Reduction

Note: Data is expressed as the percent invasion through the Matrigel Matrix and membrane relative to the migration through the Control membrane. The "Invasion Index" is also expressed as the ratio of the percent invasion of a test cell over the percent invasion of a control cell.

- a.) Determine the Percent Invasion:

$$\% \text{ Invasion} = \frac{\text{Mean \# of cells invading through Matrigel insert membrane}}{\text{Mean \# of cell migrating through control insert membrane}} \times 100$$

- b.) Determine the Invasion Index:

$$\text{Invasion Index} = \frac{\% \text{ Invasion Test Cell}}{\% \text{ Invasion Control Cell}} \times 100$$

ON 20X

NO TEST OFF 20X

TYPICAL RESULTS

The following results are typical of those obtained when the BD BioCoat™ Matrigel™ Invasion Chambers (MIC) (Cat # 354480, 24 well format) are used as described to assess the invasion of HT-1080 fibrosarcoma test cells and NIH 3T3 control cells in a 18-24 hour assay, **they are provided for reference only. Results will vary with different cell types, chemoattractants, and time.**

	HT-1080 (test cells)			NIH 3T3 (control cells)		
# cells invasion MIC (triplicate)	78	63	77	6	2	5
Mean	72.7			4.3		
# cell migration (control insert)	206	168	182	177	151	175
Mean	185.3			167.6		
% Invasion	$72.7/185.3 \times 100 =$ 39.2%			$4.3/167.6 \times 100 =$ 2.56%		
Invasion Index	$39.3\% / 2.56\% = 15.3$					

Due to the large membrane surface area (4.2 cm²/insert) of the BD BioCoat Matrigel Invasion Chambers in the 6 well format (Cat# 354481), this format may not be conducive to quantitative analysis of cell invasion for all cell types. However, the 6 well format is well suited for selecting "invasive" cell phenotypes from non-invasive cell phenotypes in response to a chemoattractant. Invaded cells are then removed from the bottom of the membrane for propagation and cell expansion. For quantitative measurements, we recommend the use of the 24-well format (Cat# 354480).

STABILITY

The BD BioCoat™ Matrigel™ Invasion Chambers are stable, for at least 3 months from date of shipment when stored at -20°C.

TECHNICAL SERVICE

For Technical Service/Questions call 1-800-343-2035.
For Customer Service call 1-800-343-2035.

REFERENCES

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NOTE: Process changes have been made to improve the performance and quality of BD BioCoat Matrigel Invasion Chambers.* Please review the following Sections for new information regarding product usage.

PRECAUTIONS (b) (page 3): New storage conditions

PROCEDURE FOR USE (page 3): Appearance of the Matrigel Basement Membrane Matrix

PROCEDURE FOR USE, Section 1.0 Rehydration (page 3) New rehydration times

PROCEDURE FOR USE, Section Invasion Studies (page 4): New suggested cell seeding densities

* US Patent pending