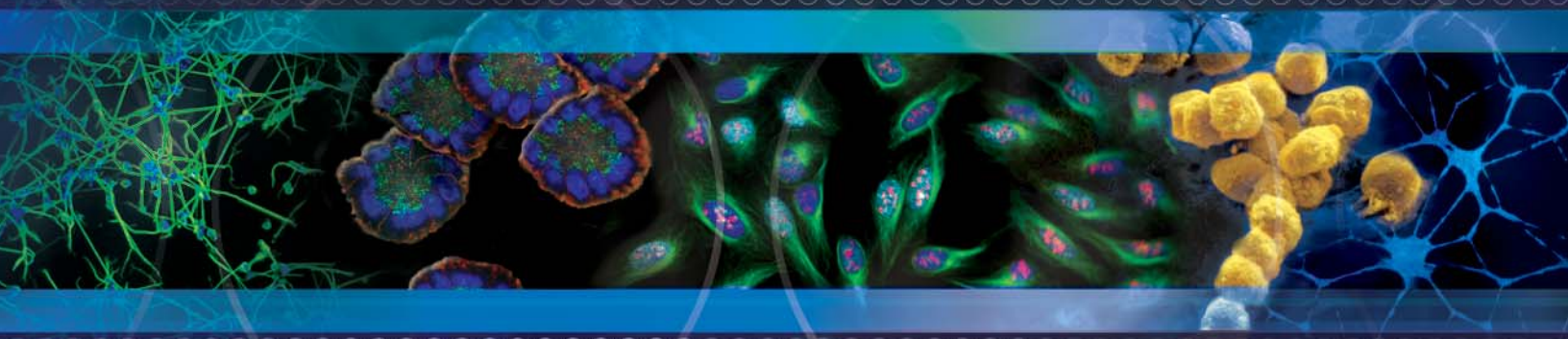


BD

Helping all people live healthy lives

# BD Biosciences
# Cell Growth and Differentiation

VWR®

Supplier Partnerships for Customer Solutions

# Table of Contents



# Cell Growth and Differentiation

## *Enhancing Cell Culture and Accelerating Discovery*

The development and normal functioning of cells depends on interactions with molecules in their microenvironment. The major classes of molecules that regulate cellular development and function include growth and differentiation factors, cell adhesion molecules, and the components of the extracellular matrix (ECM). The ECM, composed of a number of different macromolecules, influences behavior, (adherence, spreading, differentiation, and migration) and the pattern of gene expression of the cells in contact with it. To create physiologically relevant *in vitro* models that support normal cell growth and function, the components of the *in vivo* environment must be incorporated. Use of ECM proteins as coating for tissue culture surfaces permits the development of cell type specific model systems which closely mimic *in vivo* conditions.

Recognizing the increasingly important role the ECM plays in the regulation of fundamental cellular processes BD Biosciences offers a wide range of extracellular matrix proteins and attachment factors for researchers to incorporate into their cell culture systems. For over 20 years, BD has provided the research market with a wide variety of purified proteins. We were the first to offer a unique line of tissue culture vessels coated with a variety of ECM proteins and attachment factors: BD BioCoat Cellware. BD's extensive experience in protein purification, along with rigorous quality assurance testing guarantees high-quality, consistent products.

At BD Biosciences - Discovery Labware we are committed to enhancing cell culture and accelerating discovery worldwide through dedicated customer service, innovative product solutions, and technical expertise. We strive to make cell culture research more efficient and convenient for researchers by offering outstanding quality, consistency, and value.

### Commitment to Quality

We understand the importance of lot-to-lot consistency and the need for reproducible results. Through proprietary manufacturing technology, validated procedures, strict compliance with established protocols, and exacting quality control, we are able to assure the biological performance of our products as well as consistency from lot-to-lot.

### Delivering Choice

The optimal surface for cell attachment, proliferation, and differentiation is dependent on the particular cell type. BD Falcon™, BD BioCoat™, and BD™ ECM proteins provide diverse options for a variety of cells, including but not limited to commonly used cell lines such as HEK-293, primary neuronal cells, and three-dimensional culture.

### Technical Expertise

Our scientists routinely study a broad range of cells to better understand their cellular function. Our team of highly skilled and dedicated Technical Support Specialists are available to assist you in protocol development and troubleshooting.

### Customizable Solutions

We offer a custom product service to meet the unique needs of our customers. Our custom capabilities range from special package sizes and sterilization needs to barcoding and custom coating. Through our custom coating services, we will apply the coating of your choice on BD and alternative cultureware products. If you are not sure which coating you need, our Technical Support Specialists can recommend surfaces for your cell type.

# Cell Culture Surfaces

BD Biosciences offers a wide variety of surface chemistries and attachment factors appropriate for a broad range of applications. The surface of our BD Falcon™ Cultureware is rendered permanently hydrophilic via a unique vacuum-gas plasma tissue culture treatment process. This treatment process is produced in a closed, highly controlled environment ensuring a consistent treatment surface. BD Primaria™ and BD BioCoat™ surface options are ideal for enhanced cell attachment and growth of a variety of primary cells, stem cells, and transformed cell lines in serum-free or serum-containing cultures. A non-treated surface is also available for suspension or non-adherent cell culture and may also be used to study cell-cell or cell-protein interactions in an *in vitro* system.

### BD Falcon Non-treated Polystyrene

- Hydrophobic surface with low to moderate binding properties. Ideal for cell-cell or cell-protein studies.

### BD Falcon Tissue Culture-treated (TC)

- Hydrophilic surface enhances cell attachment, spreading, and cell growth by binding serum proteins to the surface. Highly controlled vacuum-gas plasma treatment creates negatively charged carboxyl groups on the polystyrene surface.
- Tested for confluency of MRC-5 cells and sterilized by gamma-irradiation.

### BD Primaria

- Supports neuronal, primary, endothelial, and tumor cells which may have difficulty attaching to or differentiate poorly on traditional TC surfaces. This surface has a unique mixture of negative and nitrogen containing positive functional groups on the polystyrene surface.
- The surface consistency of each lot is confirmed by electron spectroscopy chemical analysis (ESCA).

### BD BioCoat Poly-D-Lysine (PDL)

- Pre-coated with PDL, which promotes cell attachment of transfected and primary cells (e.g., neuronal).
- Tested for the ability to promote firm attachment of rat cerebellar granule (RCG) cells.
- Stable for six months from date of shipment at 4-30°C. Coverslips, CultureSlides, and Coverslip-Bottom Dishes stable for at least three months from date of shipment at 4°C.

### BD BioCoat Collagen I

- Pre-coated with Collagen I, derived from rat tail tendon.
- Tested for the ability to promote attachment and spreading of HT-1080 human fibrosarcoma cells.
- Stable for at least six months from date of shipment when stored at 4-30°C under dry conditions. Coverslips and CultureSlides are stable for at least three months from date of shipment when stored at 2-8°C.

### BD BioCoat Collagen IV

- Pre-coated with Collagen IV. Useful as a substrate for nerve, epithelial, endothelial, and muscle cells.
- Tested for the ability to promote attachment and spreading of PC12 rat pheochromocytoma cells or to initiate differentiation (neurite outgrowth) of NG-108 rat glioma/mouse neuroblastoma cells.
- Stable for at least three months at 2-8°C. Do not freeze.

### BD BioCoat Gelatin

- Pre-coated with Gelatin, which is commonly used for culture of vascular endothelial cells and F9 teratocarcinoma cells.
- Tested to promote proliferation of Human Umbilical Vein Endothelial Cells (HUVEC).
- Stable for at least three months from date of shipment when stored at 4-30°C under dry conditions.

### BD BioCoat Fibronectin

- Pre-coated with Human Fibronectin (HFN), which promotes cell attachment through integrin binding. HFN promotes cellular migration during wound healing and improves survival of primary cells.
- Tested to promote attachment and spreading of BHK-1 hamster kidney cells.
- Stable for at least three months at 2-8°C. Do not freeze.

### BD BioCoat Laminin

- Pre-coated with Laminin, a major component of the basement membrane used as a substrate to culture and maintain differentiated functions of a variety of cells including neuroblastoma cells and breast cancer cell lines.
- Tested for the ability to initiate neurite outgrowth of NG-108 rat glioma/mouse neuroblastoma cells.
- Stable for at least three months at 2-8°C. Do not freeze.

### BD BioCoat Laminin/Fibronectin

- Pre-coated with a combination of ECMs, which provide superior attachment and growth of glial precursor cells.
- Tested for receptor agonist induced changes in intracellular calcium-using FLUO-3 in primary rat cortical enriched cultures.
- Stable for at least three months at 2-8°C. Do not freeze.

### BD BioCoat Poly-D-Lysine/Laminin (PDL/Laminin)

- Pre-coated with a combination of ECMs, which supports neuronal differentiation of human and mouse stem cells.
- Tested for the ability to promote neurite outgrowth with primary rat cerebellar granule (RCG) cells and NG-108 rat glioma/mouse neuroblastoma cells.
- Stable for at least 3 months at 2-8°C. Do not freeze.

### BD BioCoat Poly-L-Ornithine/Laminin (PLO/Laminin)

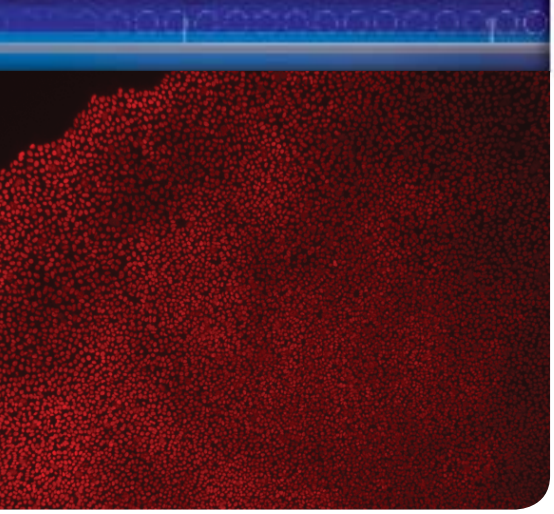
- Pre-coated with a combination of ECMs, which support growth of neuroblastoma cells and differentiation of N2a and ScN3a cells.
- Tested for the ability to promote neurite outgrowth with primary rat cerebellar granule (RCG) cells and NG-108 rat glioma/mouse neuroblastoma cells.
- Stable for at least three months at 2-8°C. Do not freeze.

### BD BioCoat Matrigel™ Matrix

- Pre-coated with solubilized basement membrane matrix extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma. Rich in ECM proteins, especially laminin, collagen IV, heparin sulphate proteoglycans, and entactin.
- Tested for the ability to promote neurite outgrowth from chick dorsal root ganglia in the absence of Nerve Growth Factor (NGF).
- Stable for at least three months at -20°C. Keep frozen until use.

| hES CELLS | HEPATOCYTES | ENDOTHELIAL CELLS | NEURONAL CELLS | EPITHELIAL CELLS | TUMOR CELLS | BD BIOSCIENCES - DISCOVERY LABWARE PRODUCT | |
|---|---|---|---|---|---|---|---|
| ■ | ■ | ■ | ■ | ■ | ■ | BD Matrigel™ Matrix | Cell Culture Reagents |
| ■ | | | | | ■ | Laminin/Entactin Complex High Concentration | |
| | ■ | ■ | ■ | ■ | ■ | Collagen I | |
| | | | ■ | | ■ | Fibronectin | |
| ■ | | | ■ | | ■ | Laminin | |
| | | | ■ | | ■ | Poly-D-Lysine | |
| | ■ | | ■ | | ■ | BD™ PuraMatrix™ Peptide Hydrogel | |
| ■ | | | | | ■ | bFGF | |
| | ■ | | | | | Hepatocyte Culture Media | |
| | ■ | ■ | ■ | ■ | ■ | ITS | |
| | | ■ | | | | Vascular Endothelial Growth Factor (VEGF) | |
| | | ■ | | | | Endothelial Cell Growth Supplement (ECGS) | |
| | | | ■ | | | Nerve Growth Factor (NGF) | |
| | | | ■ | | | Endothelial Growth Factor (EGF) | |
| | | | | ■ | | Enterocyte Differentiation Medium | |
| | ■ | | | | | Intestinal Epithelium Differentiation Media Pack | |
| | | | | ■ | | MITO+ Serum Extender | |
| | | | | ■ | | Seeding Basal Medium | |
| | | ■ | | | | HUVEC-2 | |
| | ■ | ■ | ■ | ■ | ■ | Calcein AM | |
| | ■ | ■ | ■ | ■ | ■ | $DiIC_{12}(3)$ | |
| ■ | ■ | ■ | ■ | ■ | ■ | Dispase | |
| ■ | ■ | ■ | ■ | ■ | ■ | Cell Recovery Solution | |
| ■ | | | | | | BD BioCoat™ Matrigel™ Matrix Plates for Embryonic Stem Cell Culture | Cell Culture Tools |
| | ■ | ■ | | ■ | ■ | BD BioCoat Collagen I Cellware | |
| | ■ | | | | | BD BioCoat Matrigel Matrix - for hepatocytes | |
| | | | ■ | ■ | ■ | BD BioCoat Poly-Lysine Cellware | |
| ■ | | | ■ | | ■ | BD BioCoat Laminin Cellware | |
| | | | ■ | | ■ | BD BioCoat Poly-L-Ornithine/Laminin Cellware | |
| | | | ■ | | ■ | BD BioCoat Poly-D-Lysine/Laminin Cellware | |
| ■ | ■ | ■ | ■ | ■ | ■ | BD Falcon™ Tissue Culture-treated Flasks | |
| ■ | ■ | ■ | ■ | ■ | ■ | BD Falcon CultureSlides | |
| ■ | ■ | ■ | ■ | ■ | ■ | BD Falcon 96-well Imaging Plates | |
| ■ | ■ | ■ | ■ | ■ | ■ | BD Primaria™ Cultureware | |
| | ■ | | | | | Cholyl-lysyl-Fluorescein (CLF) | Hepato-cytes |
| | ■ | | | | | BD Gentest™ Hepatocytes | |
| | ■ | | | | | Hepatocyte Differentiation Environment | Cell Environments |
| | | ■ | | | | Endothelial Cell Growth Environment | |
| | | ■ | | | | BD BioCoat Angiogenesis System: Endothelial Cell Tube Formation | |
| | | ■ | | | | BD BioCoat Angiogenesis System: Endothelial Cell Migration | |
| | | ■ | | | | BD BioCoat Angiogenesis System: Endothelial Cell Invasion | |
| | | | | ■ | | BD BioCoat Intestinal Epithelium Differentiation Environment | |
| | | | | ■ | | BD BioCoat HTS Caco-2 Assay System | |
| | | | | | ■ | BD BioCoat Matrigel Invasion Chamber | |
| | | | | | ■ | BD BioCoat Tumor Invasion System | |
| | | | | ■ | | BD BioCoat Fibrillar Collagen Cell Culture Inserts | Membrane Insert Systems |
| | | | | ■ | | BD BioCoat Fibrillar Collagen 24-Multiwell Insert System | |
| ■ | ■ | ■ | ■ | ■ | ■ | BD BioCoat and BD Falcon Inserts | |

*For guideline use only. This is not a complete list of all applications for these products.*



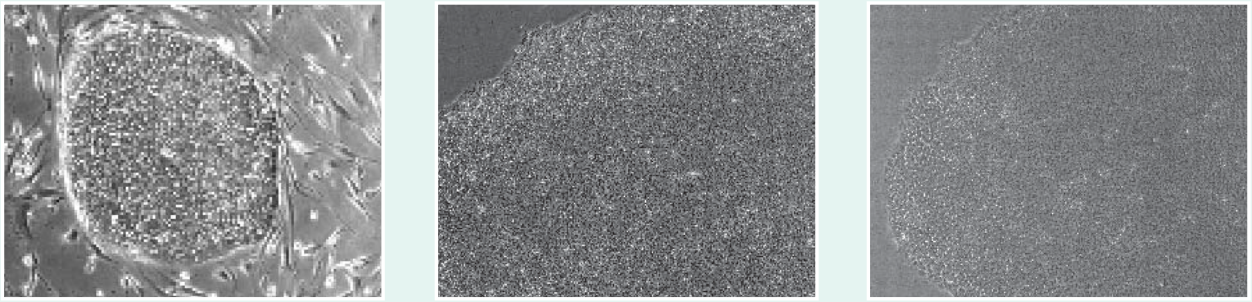
# Human Embryonic Stem Cells

Human embryonic stem (hES) cells are pluripotent cells derived from the inner cell mass of a blastocyst. These cells can either self-renew, thereby maintaining their pluripotency, or differentiate into all three germ layers depending upon the culture conditions. Induced pluripotent stem (iPS) cells, which are similar in potential to hES cells, have been generated by infecting adult cells. iPS cells, like hES cells, can form all three germ layers as well as self-renew. Tremendous hope is associated with the potential application of hES and iPS cells in cell therapy and regenerative medicine because of their ability to differentiate into multiple, clinically useful cell types. Defined culture conditions are essential to realizing the potential of hES and iPS cells.

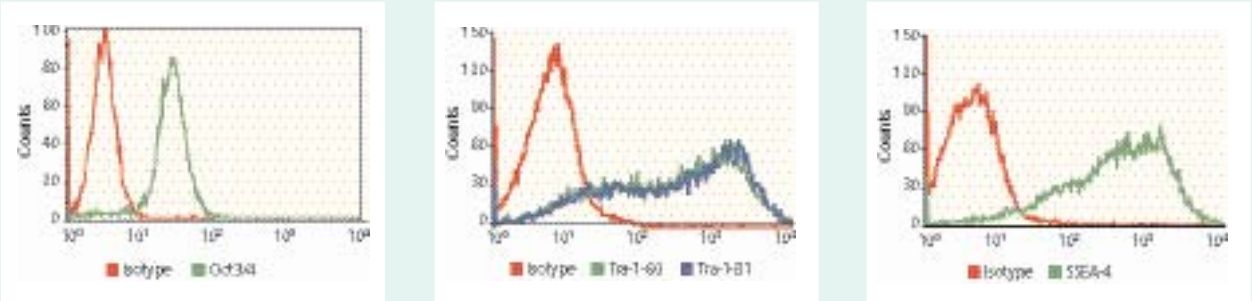
A culture environment for hES cells consisting of both a serum-free, defined medium, and a cell culture surface specifically qualified for hES cells saves researchers time and resources normally spent qualifying reagents. BD Biosciences, StemCell Technologies, and the WiCell™ Research Institute have established a strategic collaboration to develop optimized, feeder-independent cell culture environments for hES cell research, including serum-free, defined media and qualified surfaces. BD Matrigel™ Matrix, coupled with a variety of culture media, has been widely accepted as an alternative substrate to feeder-dependent culture of hES cells[1-4], and BD Matrigel Matrix has been used to culture iPS cells[5-6]. BD Matrigel Matrix is a reconstituted basement membrane isolated from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma.

FIGURE 1 • HUMAN EMBRYONIC STEM CELLS CULTURED ON BD MATRIGEL hESC-QUALIFIED MATRIX

**A.**



**B.**




1A. Phase contrast images of H9 colonies grown on mouse embryonic fibroblast (MEF) feeder layer in hES media (left), BD Matrigel hESC-qualified Matrix in MEF-conditioned media (middle), or mTeSR™1 maintenance media (right). Images were taken at 4x magnification.

1B. Flow cytometry analysis of H9 cells cultured on BD Matrigel hESC-qualified Matrix coated surface in mTeSR1 maintenance media. Cells were probed with the following antibodies: Tra-1-60 PE (Cat. No. 560193), Tra-1-81 PE (Cat. No. 560161), SSEA-4 PE (Cat. No. 560128) and Oct3/4 PE (Cat. No. 560186) compared to isotype control. Percent positive is indicated. Cells were run on a BD FACSCalibur™ system and the data was analyzed with CellQuest software.

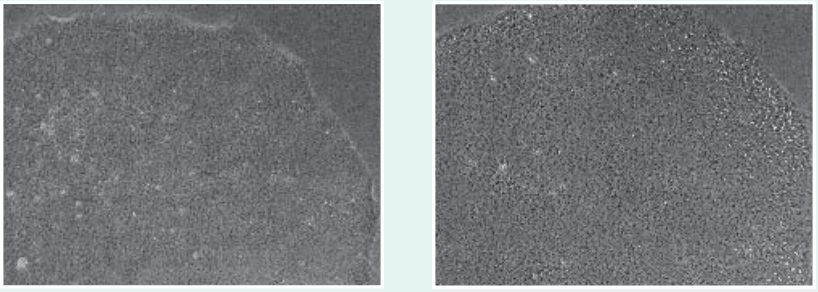
StemCell Technologies has commercially developed and optimized WiCell™ Research Institute's mTeSR™1 medium formulation to standardize feeder-independent hES cell culture. mTeSR1 is complete, defined and serum-free, and has been designed to maintain and expand hES cells in an undifferentiated state when used with BD Matrigel™ hESC-qualified Matrix as a substrate (**Figure 1**).

An alternative surface for hES cell culture is BD™ Laminin/Entactin Complex High Concentration (**Figure 2**). BD Laminin/Entactin Complex High Concentration, with a purity greater than or equal to 90%, is a more defined surface that can support undifferentiated hES cell growth. Unlike BD Matrigel hESC-qualified Matrix, this surface is not specifically qualified for maintenance of undifferentiated hES cells.

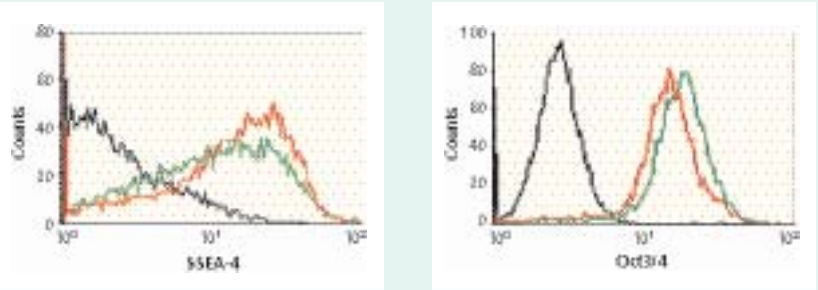
FIGURE 2 • BD LAMININ/ENTACTIN COMPLEX HIGH CONCENTRATION FOR HUMAN EMBRYONIC STEM CELL CULTURE

**A. BD Matrigel hESC-qualified Matrix**

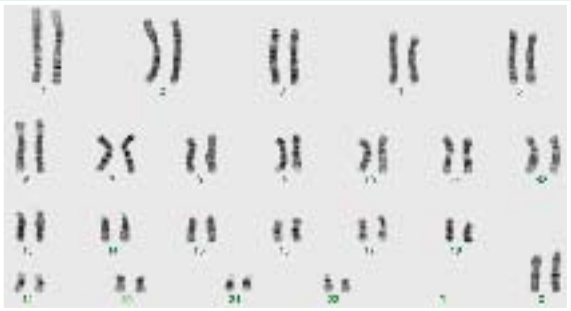
**BD Laminin/Entactin Complex High Concentration**



**B.**




**C.**



2A. Phase contrast images of H9 cells grown on BD Matrigel hESC-qualified Matrix (left) and BD Laminin/Entactin Complex High Concentration (right) in mTeSR1 maintenance media. Images were taken at 4x magnification.

2B. Flow cytometry analysis of H9 cells cultured on BD Laminin/Entactin Complex High Concentration (red line) and BD Matrigel hESC-qualified Matrix coated surface (green line) in mTeSR1 maintenance media. Cells were probed with the following antibodies: SSEA-4 PE (Cat. No. 560128) and Oct3/4 PE (Cat. No. 560186) compared to isotype control (black line). Cells were run on a BD FACSCalibur™ system and the data was analyzed with CellQuest software. Both surfaces supported undifferentiated expansion of hESC, H9.

2C. G banding chromosome analysis. Karyotype analysis of H9 cells grown on BD Laminin/Entactin Complex High Concentration in mTeSR1 media for 26 passages. Cells maintained normal karyotype under these culture conditions.

## Tools for Human Embryonic Stem Cell Culture

| Cat. No. | Description | Qty. |
|---|---|---|
| **Cell Culture Reagents** | | |
| **Extracellular Matrix Proteins** | | |
| 80085-668 | Laminin/Entactin Complex High Concentration | 10.5 mg |
| **Cytokines and Media Addtives** | | |
| 47743-574 | bFGF, human recombinant | 10 µg |
| **Cell Recovery Reagents** | | |
| 47743-724 | Dispase | 100 ml |
| 47743-696 | Cell Recovery Solution | 100 ml |
| **Cell Culture Tools** | | |
| **BD BioCoat™ Matrigel™ Matrix Plates for Embryonic Stem Cell Culture** | | |
| 89022-350 | 6-well Plates | 5 |
| **BD Falcon™ Multiwell Cell Culture Plates** | | |
| 62406-161 | 6-well Flat-bottom with lid, Tissue Culture-treated | 1 |

**For a complete product listing, see page 19.**

### *DID YOU KNOW?*

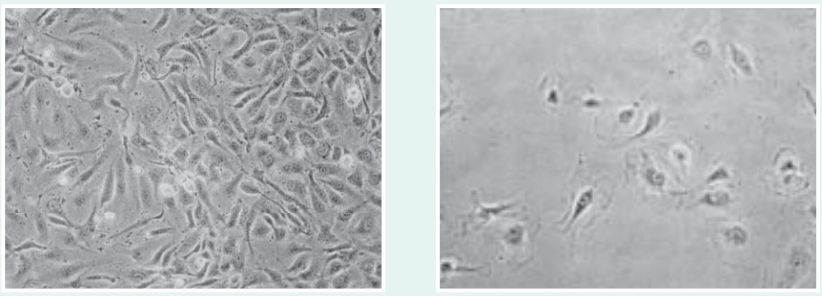
- BD Biosciences offers a full range of pipets and tubes. Please contact your sales representative for more information.

# Endothelial Cells

Endothelial cells are a specialized type of epithelial cell which forms the inner layer of blood vessels. These cells play a key role in angiogenesis, the development of new blood vessels from pre-existing vessels. Angiogenesis is a multi-step process that is important for both physiological and pathological development. During angiogenesis, endothelial cells are activated and express matrix metalloproteinases (MMPs), which degrade the vascular basement membrane. In response to environmental cues, endothelial cells secrete MMPs and then invade through the basement membrane to form new capillary networks.

Endothelial cells are tested in a variety of assays for functions that contribute to the angiogenesis process. Collagen I coated surfaces are suitable for culturing endothelial cells such as fetal bovine heart endothelial cells (FBHECs) and human umbilical vein endothelial cells (HUVECs) (**Figure 3**). *In vitro* assays of endothelial cell function include cell migration[7], invasion[8], and tubule formation[9-15]. Both the BD BioCoat™ Angiogenesis System: Endothelial Cell Invasion and the BD BioCoat Angiogenesis System: Endothelial Cell Migration allow for rapid data collection without multiple handling steps. These quantitative assays utilize BD FluoroBlok™ microporous polyethylene terephthalate (PET) membranes (3 µm pore size) which effectively block the fluorescence signal from labeled cells that have not invaded or migrated through the membrane, respectively, thereby allowing the selective detection of cells that reside on the underside of the membrane (**Figure 4**). To perform fluorescence detection, cells may be pre-labeled or post-labeled with a fluorescent dye (**Figure 5**). The pre-labeling technique enables real-time kinetic measurements of cell migration or invasion. Endothelial cells must be able to migrate and enzymatically degrade the basement membrane in order for angiogenesis to occur. The wells of BD BioCoat Angiogenesis System: Endothelial Cell Invasion are evenly coated with BD Matrigel™ Matrix, which allows researchers to examine the ability of endothelial cells to invade through reconstituted basement membrane in response to chemoattractants, such as VEGF, in the presence or absence of anti-angiogenic agents (**Figure 6**).

FIGURE 3 • EFFECTS OF BD BIOCOAT ENDOTHELIAL CELL GROWTH ENVIRONMENT ON HUVEC

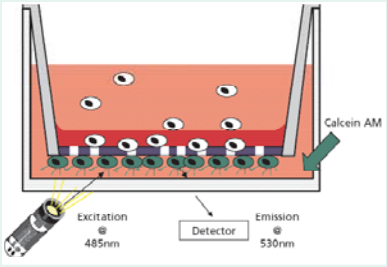



BD BioCoat Endothelial Cell Growth Environment utilizes BD BioCoat Collagen I Cellware and BD™ Endothelial Cell Culture Medium to enhance endothelial attachment and proliferation. HUVECs grown for five days using the BD BioCoat Endothelial Cell Growth Environment form a confluent monolayer and show numerous mitotic cells (A). HUVECs grown for five days in basal medium containing 10% FBS on tissue culture-treated plastic show sparse growth (B).

### *DID YOU KNOW?*

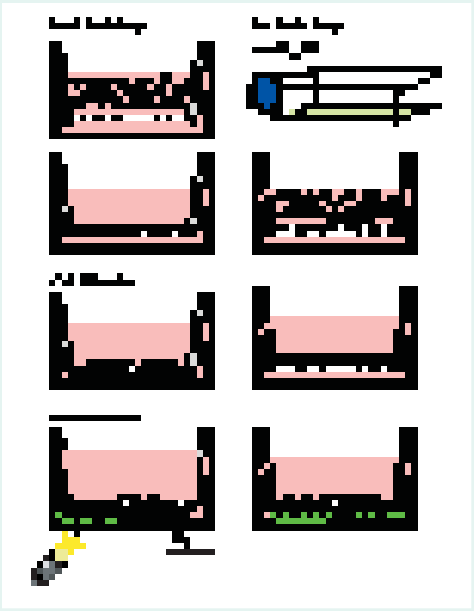
- The use of BD™ Cell Recovery Solution or BD Dispase is necessary to recover cells cultured on BD Matrigel™ Matrix.

## FIGURE 4 • LABELING CELLS POST-INVASION WITH CALCEIN AM



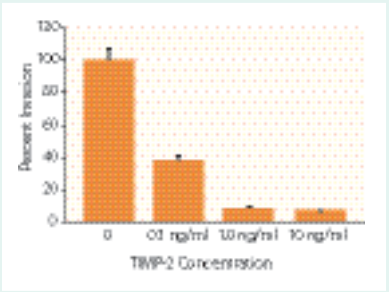

A fluorescence plate reader quantifies cells post-invasion by measuring fluorescence which correlates to cell number. Cells on top of the BD FluoroBlok™ membrane are not detected by a bottom-reading fluorometer.

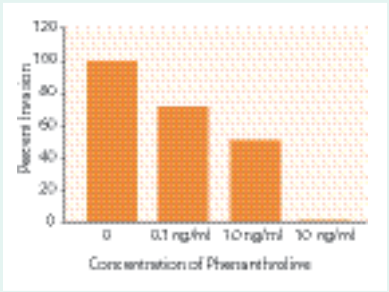
## FIGURE 5 • LABELING METHODS FOR ENDPOINT OR REAL-TIME KINETIC MIGRATION AND INVASION ASSAYS



BD FluoroBlok Inserts can be used for endpoint or real-time kinetic assays. For endpoint assays, the cell migration or invasion assay is performed with unlabeled cells. At the end of the assay the cells are labeled with a fluorescent dye, such as BD™ Calcein AM, and the data is collected using a bottom reading fluorescent plate reader. For real-time kinetic assays, the cells are pre-labeled with a fluorescent dye, such as BD $DiIC_{12}(3)$. After labeling, the migration or invasion assay is run with data collected over a time course using a bottom reading fluorescent plate reader.

## FIGURE 6 • EFFECTS OF TIMP-2 AND 1'10' PHENATHANTHROLINE IN VEGF-MEDIATED HMVEC INVASION




Human microvascular endothelial cells (HMVECs) were assayed in the BD BioCoat Angiogenesis System: Endothelial Cell Invasion in the presence of VEGF (4 µg/ml) with varying concentrations of (left) TIMP-2 or (right) 1'10' phenanthroline in the bottom chamber. Cells were allowed to invade for 22 ± 1 hour. Cells were labeled post-invasion with BD Calcein AM (4 µg/ml) and then analyzed for invasion through BD Matrigel™ Matrix using an Applied Biosystems CytoFluor® 4000 plate reader [485/540 nm (Ex/Em) wavelengths]. Data represents the mean of n=3 inserts ± S.D.

## Tools for Endothelial Cell Culture

| Cat. No. | Description | Qty. |
|---|---|---|
| **Cell Culture Reagents** | | |
| **Extracellular Matrix Proteins** | | |
| 44743-720 | BD Matrigel Basement Membrane Matrix Growth Factor Reduced | 10 ml |
| **Cell Recovery Reagents** | | |
| 47743-724 | Dispase | 100ml |
| 47743-696 | Cell Recovery Solution | 100 ml |
| **Fluorescent Dyes** | | |
| 89044-506 | $DiIC_{12}(3)$ | 100 mg |
| 89044-502 | Calcein AM | 10 x 50 µg |
| **HUVEC Cells** | | |
| BD354151 | HUVEC-2 Cells | 1 cryovial |
| **Specialty Media** | | |
| 62405-070 | Endothelial Cell Culture Media | 500 ml |
| **Cytokines and Media Additives** | | |
| 62405-784 | Endothelial Cell Growth Supplement, bovine | 15 mg |
| 47743-610 | Vascular Endothelial Growth Factor, human recombinant | 10 µg |
| **Cell Culure Tools** | | |
| **BD BioCoat Collagen I Cellware** | | |
| 62405-617 | 100 mm Dish | 10 |
| **Cell Environments** | | |
| **BD BioCoat Cell Environment** | | |
| 62405-590 | Endothelial Cell Growth Environment | 1 |
| **Membrane Insert Systems** | | |
| **BD BioCoat Angiogenesis System: Endothelial Cell Migration** | | |
| 47743-788 | 24-Multiwell Insert Plate with lid | 1 |
| **BD BioCoat Angiogenesis System: Endothelial Cell Invasion** | | |
| BD354141 | 24-Multiwell Insert Plate with lid | 1 |
| **BD BioCoat Angiogenesis System: Endothelial Tube Formation** | | |
| 47745-226 | 96-Multiwell Insert Plate with lid | 1 |

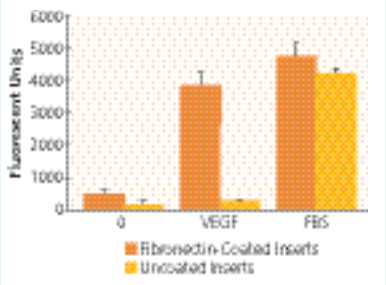
**For a complete product listing, see page 19.**

BD BioCoat™ Angiogenesis System: Endothelial Cell Migration consists of BD FluoroBlok™ inserts evenly coated with human fibronectin (**Figure 7**). Studies conducted using the post-labeling technique demonstrated that BD™ HUVEC-2 cells migrate towards VEGF in a concentration dependent manner (**Figure 8**).

During angiogenesis, endothelial cells form capillaries once they have invaded through the basement membrane. The correct culture surface is critical for successful endothelial cell tube formation *in vitro*.

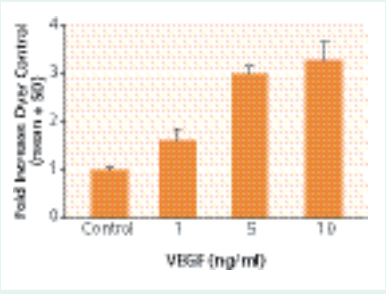
* BD BioCoat Angiogenesis System: Endothelial Cell Tube Formation offers a standardized and robust assay for studying endothelial cell tubulogenesis. For customers interested in establishing an assay for tube formation using vialed BD Matrigel Matrix, we recommend pre-testing lots to ensure optimal performance.

## FIGURE 7 • HUVEC MIGRATION ON UNCOATED AND HUMAN FIBRONECTIN-COATED INSERTS




Migration assays were conducted using HUVECs in the BD BioCoat Angiogenesis System: Endothelial Cell Migration and compared with uncoated BD FluoroBlok 24-Multiwell Inserts using both FBS (5%) and VEGF (10 µg/ml) as chemoattractants. The cells were allowed to migrate for 22 ± 1 hour. Cells were labeled post-migration with Calcein AM (4 µg/ml) and measured by detecting the fluorescence of the cells that migrated through the BD FluoroBlok membrane using an Applied Biosystems CytoFluor® 4000 plate reader [485/530 nm (Ex/Em) wavelengths]. The results indicate a marked increase in migration in response to VEGF when the assay was performed on the fibronectin-coated inserts included in the system. Data represents the mean of n=3 inserts ± S.D.

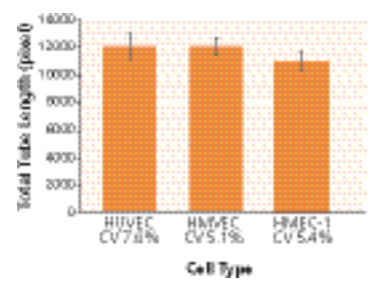
## FIGURE 8 • BD HUVEC-2 CELLS EXHIBIT CONCENTRATION-DEPENDENT MIGRATION TOWARDS VEGF




BD HUVEC-2 cells assayed in the BD BioCoat Angiogenesis System: Endothelial Cell Migration (96-Multiwell format) in response to increasing concentrations of VEGF. Samples were incubated for 22 hours. Cells were labeled post-migration with BD Calcein AM and measured by detecting the fluorescence of cells that migrated through the fibronectin-coated BD FluoroBlok membrane with the Victor2™ plate reader (PerkinElmer) at 485 nm emission. Data represents the mean of n=4 inserts ± S.D.

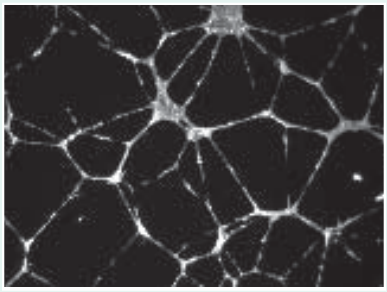
Both primary endothelial cells and endothelial cell lines have been demonstrated to form tubules on the BD BioCoat™ Angiogenesis System: Endothelial Cell Tube Formation (**Figures 9-11**) which is comprised of a 3D gel of BD Matrigel Matrix. The BD BioCoat Angiogenesis Systems are available in 24- and 96-Multiwell formats, which can be used for moderate to high throughput compound screening. BD Matrigel™ Matrix has also been extensively used to study *in vivo* angiogenesis[10-11, 16-18] as a less technically challenging alternative to the corneal implantation model. A "plug" of material is placed subcutaneously, followed by histological quantification 7-10 days later. These *in vitro* and *in vivo* assays give researchers multiple options for exploring endothelial cell functions that are essential during angiogenesis.

## FIGURE 9 • HUMAN ENDOTHELIAL CELL TYPES EXHIBIT TUBE FORMATION



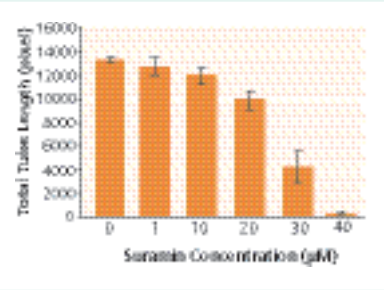

HUVEC, HMVEC, and the human endothelial cell line HMEC-1 exhibit tube formation on BD BioCoat Angiogenesis System: Endothelial Cell Tube Formation. For this study, 20,000 cells of each cell type were added to wells containing pre-solidified BD Matrigel Matrix. The assay was incubated for 18 hours. Each bar represents the mean of n=32 wells ± S.D.

## FIGURE 10 • CONFOCAL IMAGE OF BD™ HUVEC-2 CELL TUBE FORMATION



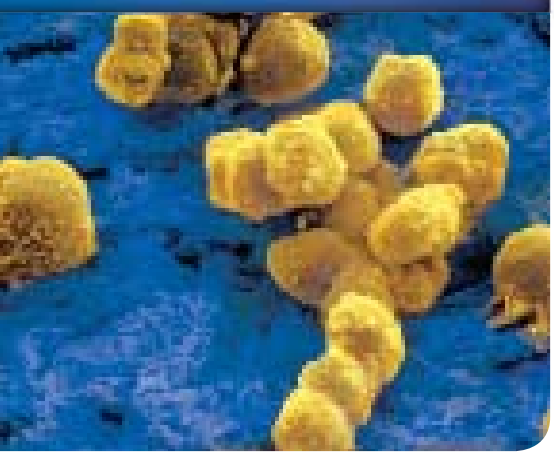
BD HUVEC-2 cells were assayed using the BD BioCoat Angiogenesis System: Endothelial Cell Tube Formation. Cells were stained using BD Calcein AM. Confocal images were captured using the BD Pathway™ Bioimager in confocal mode using the 4x objective (NA 0.13) for quantification of tubule formation.

## FIGURE 11 • SURAMIN INHIBITS HMEC-1 TUBE FORMATION




HMEC-1 cells (40,000 cells/ml) were treated with Suramin at concentrations ranging from 0-40 μm and then analyzed for tube formation using BD BioCoat Angiogenesis System: Endothelial Cell Tube Formation. 50 µl of cells plus compound were added to wells containing pre-solidified BD Matrigel Matrix. Samples were incubated at 37°C, 5% $CO_2$ for 18 hours before staining with BD Calcein AM. Images were acquired with a 2x objective lens and the total tube length was measured using MetaMorph® (Universal Imaging Corporation™). Each bar represents the mean of n=8 wells ± S.D.

# Hepatocytes

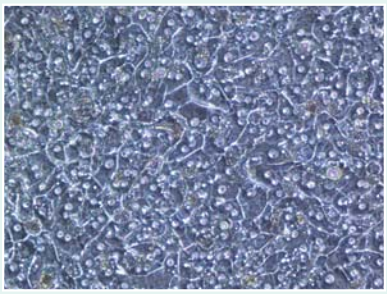


Pseudo-colored image for illustrative purposes only.

Hepatocytes are liver epithelial cells used for both basic research and drug metabolism studies. Fresh and cryopreserved primary hepatocytes contain all the major enzyme pathways for drug and xenobiotic biotransformation. These include the major phase I drug metabolism enzyme family (P450) and phase II enzymes (UGT, SULT, GST and NAT). Hepatocytes also contain all the gene regulation pathways for P450 induction. Appropriate culture conditions are required to maintain hepatic P450 activity.

Hepatocytes can be cultured on Collagen I[19-22], BD Matrigel™ Matrix[23-27] or BD™ PuraMatrix™[28-29]. BD BioCoat™ Collagen I Cellware is a commonly used surface for cultures of both fresh and cryopreserved hepatocytes[30-31] (**Figure 12**). Cells cultured on this surface maintain their biological activity, as shown by P450 induction (**Figure 13**). Sandwich cultures, such as hepatocytes grown on BD BioCoat Collagen I with BD Matrigel Matrix overlay, are used to assess bile canaliculi formation[32]. Choly-lysyl-fluorescein (CLF) is a fluorescein-labeled bile acid that is secreted into bile canaliculi by bile salt export pump (BSEP) which can be used to visualize bile canaliculi (**Figure 14**). BD Matrigel Matrix has been shown to suppress cell growth and prevent growth-associated dedifferentiation[23], as well as maintain liver-specific functions *in vitro* longer than most collagen-based systems[24-26]. Hepatocytes cultured on BD Matrigel Matrix also have a more differentiated morphology than hepatocytes cultured on collagen I (**Figure 15**). Both BD Collagen I and BD Matrigel Matrix are animal-derived products; BD PuraMatrix, a synthetic peptide hydrogel, is a suitable alternative for assays that require a xeno-free culture environment. Therefore the appropriate culture surface depends on the experimental goals (e.g., drug metabolism, bile canaliculi formation or xeno-free environment).

FIGURE 12 • BD INDUCIBLE CRYOPRESERVED HUMAN HEPATOCYTES CULTURED ON BD BIOCOAT COLLAGEN I



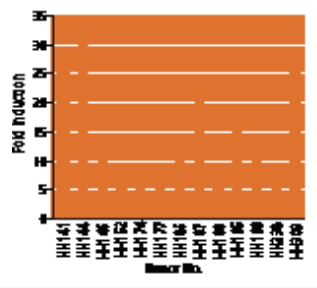
BD Gentest™ Inducible-qualified Human CryoHepatocytes were isolated using the BD Gentest CryoHepatocyte Purification Kit and resuspended in freshly prepared ISOMs seeding media at a concentration of $1x10^6$ cells/ml. Cells were plated onto BD BioCoat Collagen I 24-well plates and incubated for approximately 2 hours, after which plating media was removed and replaced with supplemented BD Hepatocyte Culture Media.

### *DID YOU KNOW?*

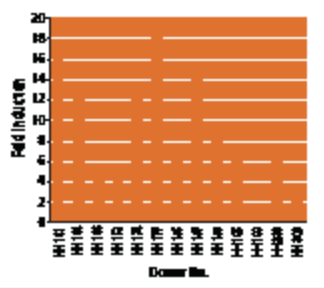
- BD Biosciences offers a custom barcoding service. This service provides high-quality barcode labels affixed to any side of a microplate.

## FIGURE 13 • INDUCTION OF BD GENTEST™ INDUCIBLE-QUALIFIED HUMAN CRYOHEPATOCYTES

**A. CYP3A4**

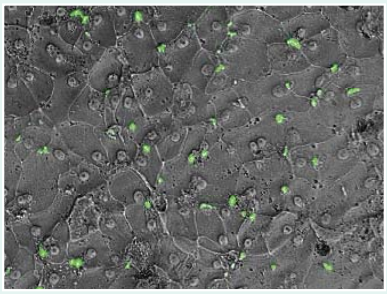



**B. CYP1A2**




BD Gentest Inducible-qualified Human CryoHepatocytes were isolated using the BD Gentest CryoHepatocyte Purification Kit and resuspended into freshly prepared ISOMs seeding media at a concentration of 1x10$^6$ cells/ml. Cells were plated onto BD BioCoat™ Collagen I 24-well Multiwell Plates and incubated for approximately 2 hours, after which plating media was removed and replaced with supplemented BD™ Hepatocyte Culture Media. Cells were monitored for degree of attachment at 18-24 hours after plating and daily during the experiment. Cells were induced with either 20 µM Rafampicin (A) or 20 µM β-Napthoflavone (B) over a 3-day period. Controls were treated with the appropriate solvent control. Metabolic activity was determined on day 5 of the experiment using 200 µM Testosterone as a substrate to measure CYP3A4 activity and 100 µM Phenacetin as a substrate for CYP1A2. Assays were run for 30 minutes and 60 minutes, respectively. Analysis was performed by HPLC and activity expressed per mg of protein.

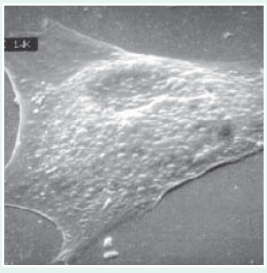
## FIGURE 14 • BD GENTEST CHOLY-LYSYL-FLUORESCEIN SEQUESTERED IN BILE CANALICULI



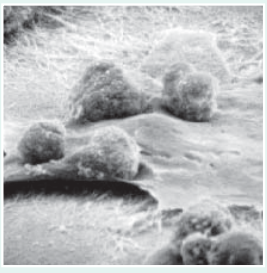
CLF sequestered in the bile canaliculi of BD Gentest Inducible-qualified Human CryoHepatocytes cultured on BD BioCoat Collagen I overlaid with BD Matrigel Matrix.

## FIGURE 15 • EFFECTS OF ECM ON CELL MORPHOLOGY: MICROGRAPHS OF HEPATOCYTES CULTURED ON VARIOUS CULTURE SUBSTRATA

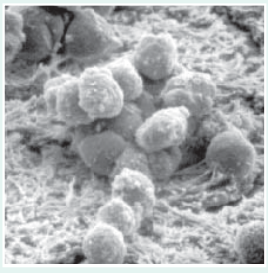
**A.**



**B.**



**C.**

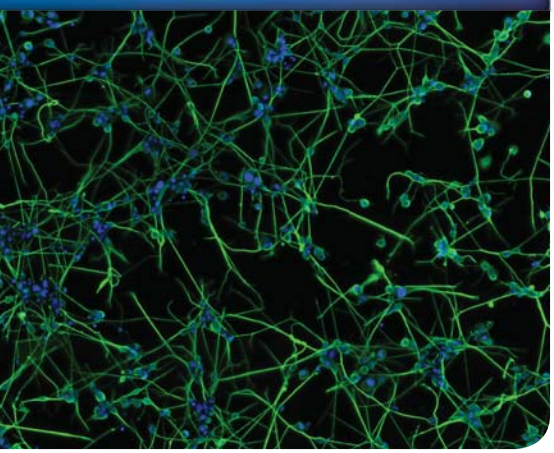


Scanning electron micrographs of primary rat hepatocytes cultured for two days on collagen I (A), collagen I gel (B), or BD Matrigel Matrix (C). Note the clusters of spherical cells for hepatocytes cultured on BD Matrigel Matrix, typical of differentiated cells.

## Tools for Hepatocyte Cell Culture

| Cat. No. | Description | Qty. |
|---|---|---|
| **Cell Culture Reagents** | | |
| **Hepatocyte Culture Media Kit** | | |
| 62405-070 | Hepatocyte Culture Media Kit | 500 ml |
| **Extracellular Matrix Proteins** | | |
| 47743-656 | Collagen I, rat tail | 100 mg |
| 47743-716 | BD Matrigel Matrix, phenol red-free | 10 ml |
| **Cell Recovery Reagents** | | |
| 47743-724 | Dispase | 100 ml |
| 47743-696 | Cell Recovery Solution | 100 ml |
| **Cell Culture Tools** | | |
| **BD BioCoat™ Collagen I** | | |
| 62405-601 | 6-well plates | 5 |
| **BD BioCoat Matrigel Matrix Multiwell Plates for Hepatocytes** | | |
| 62405-144 | 6-well plates | 5 |
| **Cell Environments** | | |
| **Hepatocyte Differentiation Environment** | | |
| 62405-096 | 6-well plate | 1 |

**For a complete product listing, see page 19.**

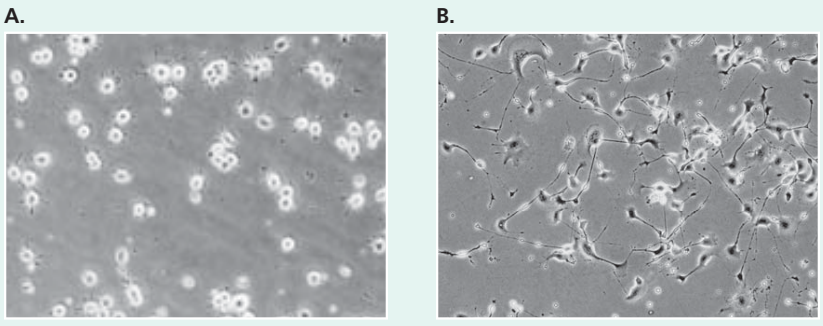


# Neuronal Cells

Neuroscience is a rapidly evolving field that encompasses a variety of cell types, including neurons and neuronal stem cells. *In vitro* culture of these diverse cell types requires appropriate culture surfaces for attachment and proliferation/differentiation, as detailed in the examples below. NG-108 rat glioma/mouse neuroblastoma cells and PC-12 cells, two neuronal cell lines, require different surfaces for attachment. NG-108 cells attach loosely to tissue culture-treated cellware, but when they are cultured on BD BioCoat™ Laminin Cellware they exhibit a more typical neuronal morphology (**Figure 16**). PC-12 cells, derived from a transplantable rat pheochromocytoma, develop neurites in response to NGF when they are cultured on collagen I (**Figure 17**). Other surfaces, including BD BioCoat Poly-D-Lysine Cellware[33] and BD BioCoat Poly-D-Lysine/Laminin[34], can also be used to culture PC-12 cells. Primary neuronal cells utilize different attachment surfaces depending on their origin and the composition of the media used during culture. Primary mouse cortical neurons and primary mouse basal forebrain cholinergic neurons have been cultured on BD BioCoat Poly-L-Lysine Cellware[35] and BD BioCoat Poly-D-Lysine/Laminin Cellware[36], respectively. Primary human neural stem cells have been grown under serum-containing conditions in tissue culture-treated BD Falcon Cell Culture Flasks[37]. Using serum-free conditions, Thonhoff, et al., showed that neuronal stem cells maintain their capacity to differentiate into both Tuj1$^+$ neuronal cells and GFAP$^+$ astroglial cells on BD™ PuraMatrix™ while differentiation of neuronal stem cells grown on BD Matrigel™ Matrix was skewed toward GFAP+ astroglial cells[38]. Both BD PuraMatrix[38-40] and BD Primaria™[41] are defined, xeno-free surfaces for 3D and 2D culture, respectively, which are compatible with neuronal cells. BD Primaria Cultureware enhances neuronal cell attachment as compared to tissue culture-treated cellware, as shown with chick embryo spinal cord neurons (**Figure 18**). These examples* illustrate the need for an appropriate growth surface which is determined by the cell type and whether a xeno-free surface with defined media is required by the experimental model.

*Other examples available in references 42-44.

FIGURE 16 • EFFECTS OF BD BIOCOAT LAMININ CELLWARE ON NG-108 NEUROBLASTOMA CELLS

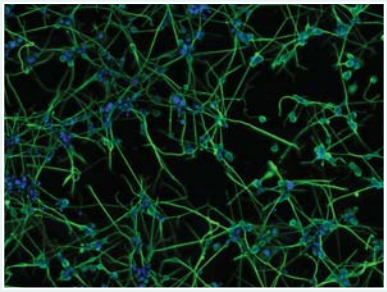



NG-108 rat glioma/mouse neuroblastoma cell morphology is surface dependent. Cells cultured on tissue culture plastic are loosely adhered and remain rounded (A). Cells cultured on BD BioCoat Laminin cellware exhibit a spindle-shaped morphology and dendritic processes (B).

### *DID YOU KNOW?*

- BD Biosciences offers a full range of 96-, 384-, and 1536-well Microplates. Custom packaging, labeling (e.g., barcoding), and custom coatings are also available. Please contact your sales representative for more information.

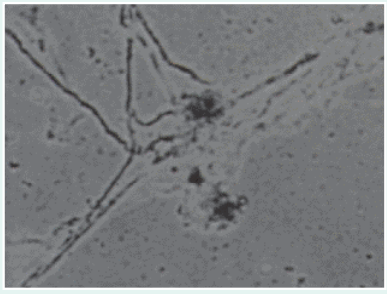
FIGURE 17 • PC12 NEURITE OUTGROWTH, CULTURED ON BD™ COLLAGEN I



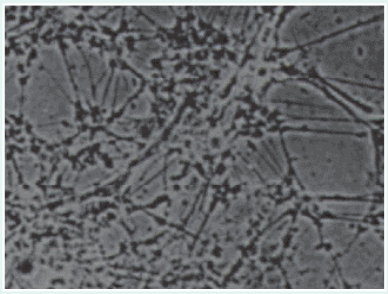
PC12 cells were maintained in DMEM with 10% FBS, 5% horse serum and 1% penicillin/streptomycin. For neurite generation, approximately 15,000 cells/well were plated in BD Falcon™ 96-well Imaging Plates that were coated with BD Collagen I, rat tail using 1.8 µg collagen per well. After 24 hours, the medium was replaced with differentiation medium (DMEM with 0.1% FBS, 0.05% horse serum, 100 ng/ml NGF). The medium was replenished every third day for 10 days. For imaging, cells were fixed with 3.7% paraformaldehyde for 20 minutes and permeabilized with 0.1% Triton-X-100 for 5 minutes. Neurites were stained with a primary mouse anti-β-tubulin antibody (Cat. No. 556321) using 0.125 µg antibody/well followed by AlexaFluor® 488 goat anti-mouse IgM at a concentration of 0.25 µg/well. Hoechst 33342 was used at 0.1 µg/well to stain the nuclei. To prevent the dissociation and fracture of fragile neuronal networks, the number of washes in the fixation and processing steps were minimized and extra care was taken in aspirating and dispensing liquids in wells. Images were acquired on a BD Pathway™ as a 4x4 montage using a 20x objective (0.75 NA).

FIGURE 18 • CHICK EMBRYO SPINAL CORD NEURONS CULTURED ON BD PRIMARIA™ CULTUREWARE

A.



B.

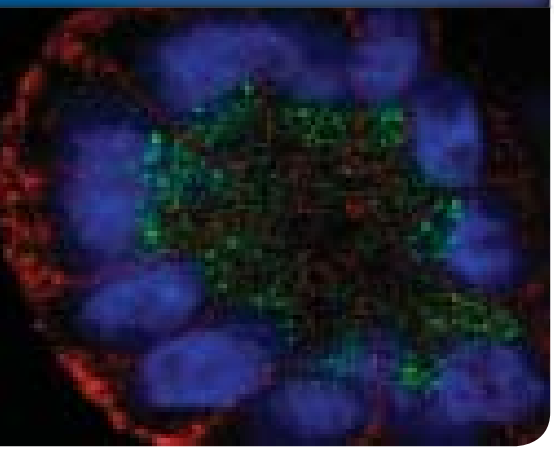


When chick embryo spinal cord neurons are cultured on BD Primaria Cultureware, growth is enhanced and extensive neurite development occurs. In this experiment, cells clumped and detached from traditional TC plates after 20 days in culture (A) but remained viable and differentiated on BD Primaria Cultureware (B).

## Tools for Neuronal Cell Culture

| Cat. No. | Description | Qty. |
|---|---|---|
| **Cell Culture Reagents** | | |
| **Extracellular Matrix Proteins** | | |
| 47743-656 | Collagen I, rat tail | 100 mg |
| 47743-728 | Fibronectin, human | 1 mg |
| 47743-734 | Laminin, mouse | 1 mg |
| 47743-715 | BD Matrigel Matrix | 10 ml |
| 47743-736 | Poly-D-Lysine | 20 mg |
| **Cytokines and Media Additives** | | |
| 47743-594 | 7S Nerve Growth Factor, mouse, natural | 100 µg |
| 47743-588 | 2.5S Nerve Growth Factor, mouse, natural | 10 µg |
| 47743-566 | Endothelial Growth Factor, human recombinant | 100 µg |
| **Cell Recovery Reagents** | | |
| 47743-724 | Dispase | 100 ml |
| 47743-696 | Cell Recovery Solution | 100 ml |
| **Cell Culture Tools** | | |
| **BD BioCoat™ Laminin Cellware** | | |
| 62405-706 | 6-well plates | 5 |
| **BD BioCoat Poly-L-Ornithine/ Laminin Cellware** | | |
| 62405-747 | 96-well plates | 5 |
| **BD BioCoat Poly-D-Lysine/ Laminin Cellware** | | |
| 62405-111 | 24-well plates | 5 |
| **BD BioCoat Poly-D-Lysine Cellware** | | |
| 62405-747 | 6-well | 5 |
| **BD Primaria Cultureware** | | |
| 25382-687 | 60 x 15 mm Dish with lid | 200 |
| **BD Falcon™ CultureSlides** | | |
| 62405-178 | 8-well | 96 |
| **BD Falcon 96-well Imaging Plate** | | |
| 89022-352 | Black/Clear, with lid | 32 |

**For a complete product listing, see page 19.**

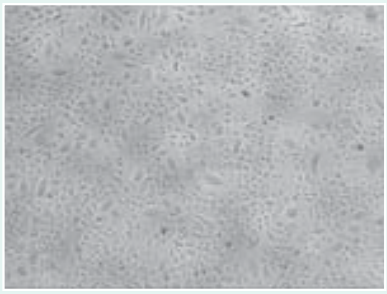


# Epithelial Cells

Epithelial cells are found throughout the body, from skin to glandular formations within tissues. *In vivo* these cells are attached to a three dimensional basement membrane matrix. The interactions between the epithelial cell and matrix proteins effect cell morphology and function. Two highly specified epithelial cell types have been discussed in the hepatocyte and endothelial cell sections, utilizing both 2-dimensional (2D) and three-dimensional (3D) culture systems. *In vitro*, 2D and 3D culture systems can be used to study different aspects of cell growth and differentiation. 2D culture systems are used for cell attachment and proliferation. 3D environments are utilized in studies requiring a more *in vivo*-like setting, such as mammary acini formation.

The BD BioCoat Cellware provides a range of 2D surfaces for cell growth. Both keratinocytes[45-46] and HEK-293[47-49] cells are examples of epithelial cells that can be studied in 2D culture environments. Keratinocytes are a major component of the epidermis; BD BioCoat Collagen I supports growth of human neonatal keratinocytes (**Figure 19**). HEK-293 cells are a human epithelial kidney cell line which exhibit enhanced attachment to poly-lysine coated surfaces as compared to tissue culture-treated surfaces. This is particularly important if the cells need to remain attached during subsequent washes (**Figure 20**). The appropriate 2D surface is determined by the cell type.

FIGURE 19 • PROLIFERATION OF HUMAN NEONATAL KERATINOCYTES ON BD BIOCOAT™ COLLAGEN I



Human neonatal keratinocytes cultured on BD BioCoat Collagen I.

Three-dimensional growth substrates can support certain cellular behaviors that are not observed when cells are cultured on a planar two-dimensional surface, as exemplified by mammary epithelial[50-54] and Caco-2[55-56] cells. *In vivo*, mammary epithelial cells form polarized acini. When tumorigenic human mammary carcinoma cells (T4-2) are cultured on a 3D substrate comprised of reconstituted basement membrane (Growth Factor Reduced BD Matrigel Matrix) they form large disorganized colonies, as shown with the T4-vector control in a study from Dr. Bissell's laboratory[51] (**Figure 21**). Epidermal growth factor receptor (EGFR) had previously been shown to be elevated in T4-2 cells, and downregulation of this signaling pathway in T4-2 cells cultured in 3D BD Matrigel™ Matrix is known to lead to phenotypic reversion to polarized acini. These cells exhibit polarized acinar architecture in the presence of the EGFR inhibitor AG1478 or when stably expressing dominant negative Rap1 (T4-DN-Rap1); reversion to a normal phenotype is shown by proper localization of α6-integrin (basal marker), β-catenin (basolateral marker) and GM130 (apical marker). These data show that three-dimensional BD Matrigel Matrix culture conditions are conducive to studying signaling pathways involved in regulating mammary acinar architecture.

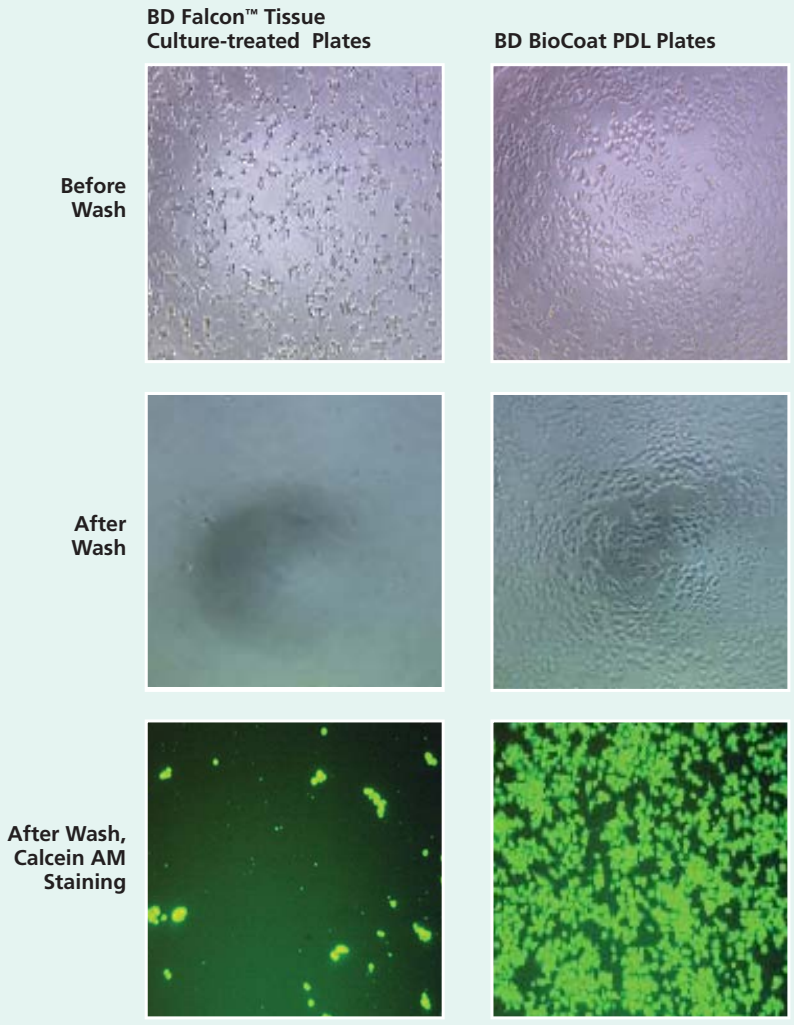
Another example of the effect of 3D growth substrates on cellular phenotypes is the use of BD BioCoat Fibrillar Collagen Inserts in Caco-2 assays. Caco-2 cells are an epithelial cell line derived from a colorectal adenocarcinoma commonly used to measure compound permeability. The gold standard for modeling drug permeability across the intestinal epithelium *in vitro* is measuring permeability across differentiated Caco-2 cells, where the cells have been cultured for 21 days on cell culture inserts. BD BioCoat HTS Caco-2 Assay System and BD BioCoat Intestinal Epithelium Differentiation Environment utilize BD BioCoat Fibrilliar Collagen Inserts and a specialized media to enhance the rate of Caco-2 differentation from 21 to 3 days (**Figures 22-23**), thereby reducing the time and labor required for the analysis of compound permeability.

The 2D and 3D cell culture systems available from BD Biosciences provide multiple options to researchers studying epithelial cells *in vitro*.

### *DID YOU KNOW?*

- BD Biosciences offers custom coatings. Please contact your sales representative for more information.

FIGURE 20 • ADHERANCE OF HEK-293 CELLS TO BD BIOCOAT™ POLY-D-LYSINE CULTUREWARE



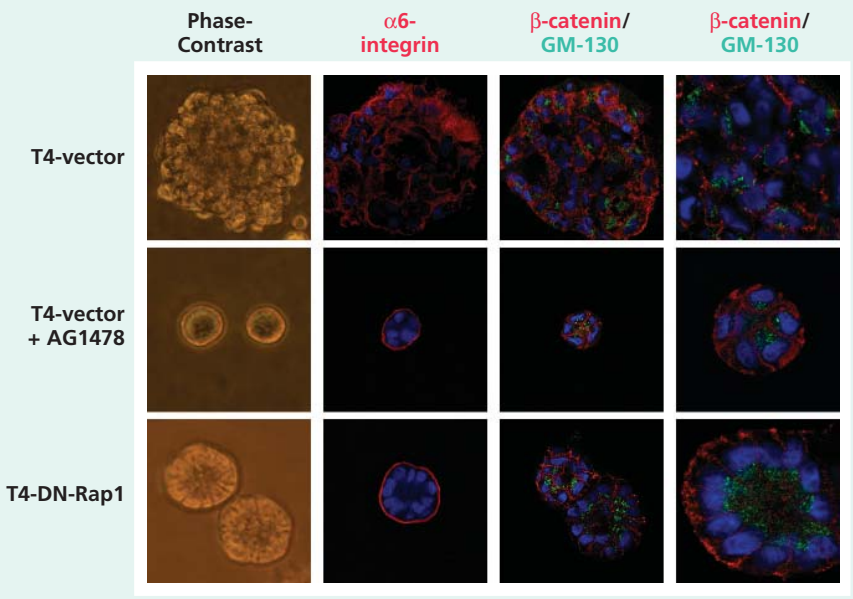

HEK-293 cells have enhanced attachment to BD BioCoat Poly-D-Lysine Cultureware as compared to BD Falcon Tissue Culture-treated Cultureware. An equal number of cells were plated on BD BioCoat Poly-D-Lysine 384-well black/clear (right) and BD Falcon Tissue Culture-treated 384-well Black/Clear Plates (left) and grown under serum-free conditions. Before washing (top), there were a similar number of cells in the BD BioCoat Poly-D-Lysine coated wells and the BD Falcon Tissue Culture-treated wells. After washing, using a Skatron Washer (Molecular Devices) (middle), the cells remained attached to the BD BioCoat Poly-D-Lysine wells while few cells remained attached to the BD Falcon Tissue Culture-treated wells. Post-wash, the cells were visualized using Calcein AM (bottom).

## Tools for Epithelial Cell Culture

| Cat. No. | Description | Qty. |
|---|---|---|
| **Cell Culture Reagents** | | |
| **Extracellular Matrix Proteins** | | |
| 47743-712 | Collagen I, rat tail | 10 x 100 mg |
| 47743-706 | BD Matrigel Matrix | 5 ml |
| **Cell Recovery Reagents** | | |
| 47743-724 | Dispase | 100 ml |
| 47743-696 | Cell Recovery Solution | 100 ml |
| **Cell Culture Tools** | | |
| **BD BioCoat™ Collagen I Cellware** | | |
| 12777-074 | 75 $cm^2$ vented-cap Flasks | 5 |
| **BD BioCoat Poly-D-Lysine Cellware** | | |
| 62406-048 | 100 mm Dishes | 10 |
| **Cell Environments** | | |
| **Intestinal Epithelium Differentiation Environment** | | |
| 62405-098 | Intestinal Epithelium Differentiation Environment | 1 |
| **BD BioCoat HTS Caco-2 Assay Systems** | | |
| 12777-040 | BD BioCoat Fibrillar Collagen 24-Multiwell Insert System plus media to perform 24 individual three-day Caco-2 assays | 1 |
| 62405-446 | Differentiation Medium | 2 x 250 ml |
| 62405-442 | Intestinal Epithelium Differentiation Media Pack | 1 kit |
| 47743-630 | MITO+ Serum Extender | 5 ml |
| 62405-444 | Seeding Basal Medium | 2 x 250 ml |
| 12777-044 | Five BD BioCoat Fibrillar Collagen 24-Multiwell Insert System plus media to perform 24 individual three-day Caco-2 assays | 1 |
| **Membrane Insert Systems** | | |
| **BD BioCoat Fibrillar Collagen Cell Culture Inserts** | | |
| 62405-119 | 1 µm inserts in four 6-well plates | 24 |

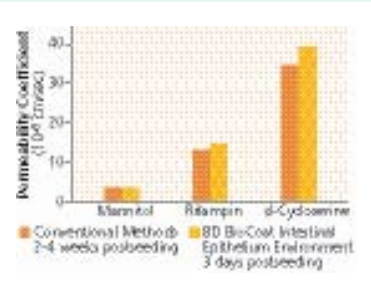
**For a complete product listing, see page 19.**

## FIGURE 21 • EFFECT OF RAP1 ACTIVITY ON T4-2 CELL POLARITY IN 3D GROWTH FACTOR REDUCED BD MATRIGEL™ MATRIX CULTURE



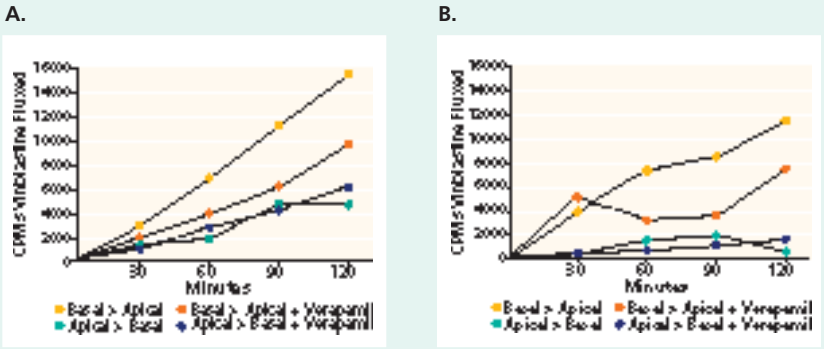

Growth Factor Reduced BD Matrigel Matrix supports mammary acini formation *in vitro*. Malignant T4-2 cells were grown in three-dimensional culture on Growth Factor Reduced BD Matrigel Matrix. Cells were stably transfected with control (T4-vector) or dominant negative-Rap1 (T4-DN-Rap1). Inhibition of EGFR with AG1478 was used as a positive control for reversion of T4-2 to normal mammary acinar architecture. Indirect immunofluorescence was used to analyze cell polarity markers for basal (α6-interin), basolateral (β-catenin) and apical (GM130) membrane domains. Bar, 5 µm. Images kindly provided by Dr. Masahiko Itoh and Dr. Mina Bissell, originally published in Cancer Research 67(10):4759-4766[51]. Reproduced with permission.

## FIGURE 22 • PERMEABILITY OF MANNITOL AND ANTIBIOTICS THROUGH CACO-2 MONOLAYERS




Barrier formation occurs three days post-seeding in the BD BioCoat Intestinal Epithelium Differentiation Environment and two to four weeks with conventional methods. Monolayers formed using either the BD BioCoat Intestinal Epithelium Differentiation Environment or conventional methods are equally permeable for each of the three compounds tested.

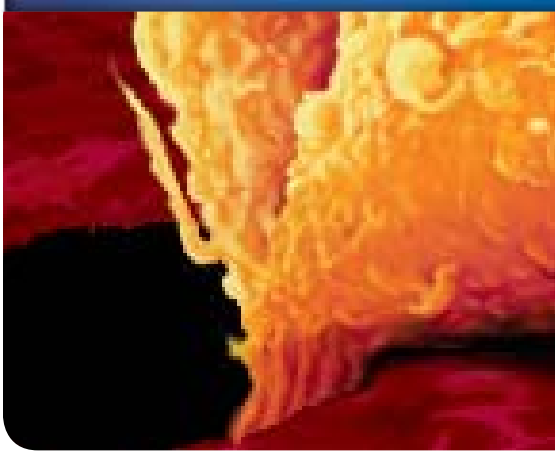
## FIGURE 23 • P-GLYCOPROTEIN (P-GP) FUNCTION IN CACO-2 CELLS




Caco-2 cells were cultured using the three-day BD BioCoat HTS Caco-2 Assay System supplemented with MITO+ Serum Extender (A) or the traditional 21-day system (B). P-gp function was assessed by adding 10 nM $^{3}$H-labeled vinblastine in PBS to either the apical or basal side of the insert. Samples were withdrawn from the non-labeled side of the insert and counted by scintillation counting. To inhibit the P-gp with verapmil, 100 µM verapamil was added to the insert chambers.

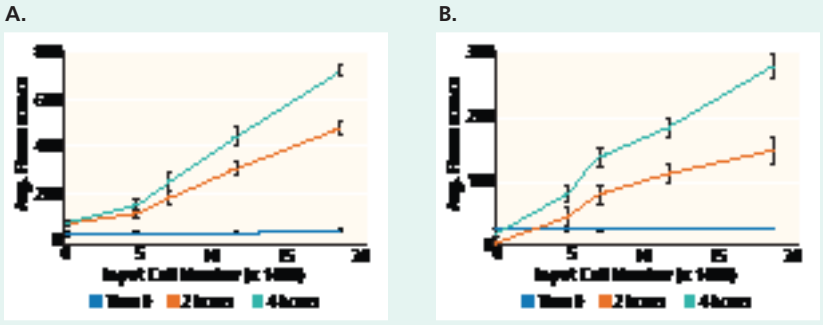
# Tumor Cells

Cancerous cells have altered cellular functions as compared to the normally functioning, non-malignant cells from which they are derived. Cell morphology and signaling pathway studies *in vitro* that incorporate the use of 3D culture systems can give insights into the effects of mis-regulated or mis-expressed proteins, as exemplified by human mammary carcinoma cells (T4-2)[51] (**Figure 20**). The hallmark of metastatic cells is their ability to invade through the basement membrane and migrate to other parts of the body. Cell migration can be studied using either BD Falcon™ Cell Culture Inserts or BD FluoroBlok™ Cell Culture Inserts for moderate to high-throughput screening (**Figure 24**). Cells must be able to both secrete proteases that break down the basement membrane as well as migrate in order to be invasive. Invasion through BD Matrigel™ Matrix-coated Cell Culture Inserts has become the gold standard for quantitative and qualitative measurement of the metastatic potential of a cell[10, 57-63]. This matrix provides a true barrier to non-invasive cells while presenting the appropriate protein structure for penetration of invading cells.



Pseudo-colored image for illustrative purposes only.

FIGURE 24 • HT-1080 MIGRATION




Migration of Calcein AM (A) and $DiIC_{12}(3)$ (B) labeled human fibrosarcoma cells (HT-1080) through BD Falcon FluoroBlok 96-Multiwell Inserts, 8 µm pore size. DMEM with 5% FCS was used as a chemoattractant in the lower wells, while DMEM/0.1% BSA was added to the control wells. The plates were incubated for four hours at 37°C, after which fluorescence of cells which had migrated through the microporous membrane was measured on the Applied Biosystems CytoFluor® 4000 and PerkinElmer HTS 7000 Plus fluorescent plate readers using excitation/emission wavelengths of 485/530 nm for Calcein AM or 530/590 nm for $DiIC_{12}(3)$. Values represent the mean of 8 wells ± S.D. Migration from as few as 4,000 input cells can be detected.

## Tools for Tumor Cell Culture

| Cat. No. | Description | Qty. |
|---|---|---|
| **Cell Culture Reagents** | | |
| **Extracellular Matrix Proteins** | | |
| 47747-220 | BD Matrigel Matrix, High Concentration | 10 ml |
| **Cell Recovery Reagents** | | |
| 47743-696 | Cell Recovery Solution | 100 ml |
| 47743-724 | Dispase | 100 ml |
| **Fluorescent Dyes** | | |
| 89044-502 | Calcein AM | 10 x 50 µg |
| 89044-506 | $DiIC_{12}(3)$ | 100 mg |
| **Membrane Insert Systems** | | |
| **BD BioCoat Matrigel Invasion Chambers** | | |
| 62405-744 | 8.0 µm inserts in two 24-well plates | 24 |
| **BD BioCoat Tumor Invasion System** | | |
| 47733-148 | One insert plate with 24-well plate and lid | 1 |
| **BD BioCoat Cell Culture Inserts** | | |
| 62405-722 | Laminin, 3 µm inserts in two 24-well plates | 24 |
| **BD Falcon Cell Culture Inserts** | | |
| 62406-226 | 3 µm pore size with 24-well plate and lid | 1 |

**For a complete product listing, see page 19.**

### *DID YOU KNOW?*

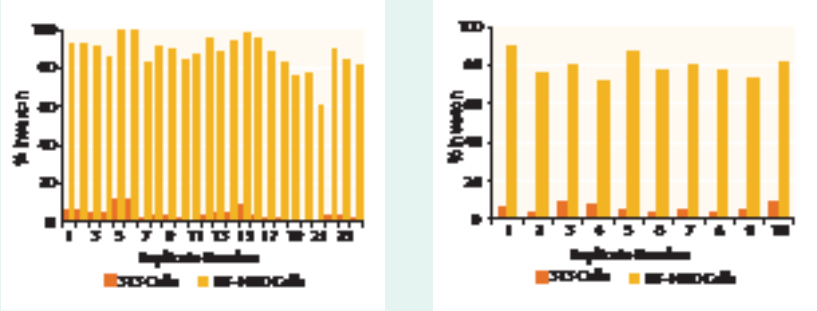
- BD Biosciences offers a full range of dishes and flasks. Please contact your sales representative for more information.

The BD BioCoat™ Matrigel Invasion Chambers and BD BioCoat Tumor Invasion Systems are optimized systems that utilize standardized coating procedures to ensure even coating of BD Matrigel™ Matrix for reproducible results (**Figure 25**). The BD BioCoat Tumor Invasion System provides a unique, quantitative platform that can be used to determine the effects of anti-metastatic compounds on invasive cell types (**Figure 26**). For *in vivo* studies, BD Matrigel Matrix can be used to help support tumor cell engraftment in mice[64-66]. These tools allow researchers to dissect various areas of tumor biology, from analysis of signaling pathways *in vitro* to *in vivo* tumor formation.

FIGURE 25 • COMPARISON OF MEAN PERCENT INVASION

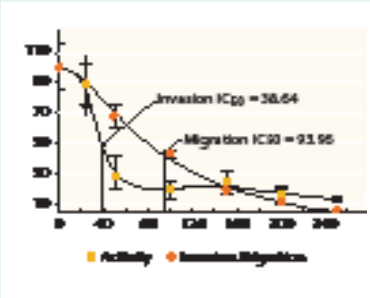
**A. BD BioCoat 96-Multiwell Tumor Invasion System**

**B. BD BioCoat 24-Multiwell Tumor Invasion System**



Multiple lots of the BD BioCoat 96-Multiwell Tumor Invasion System and BD BioCoat 24-Multiwell Tumor Invasion System were assayed to show reproducibility with these systems. Multiple lots of BD BioCoat 96-Multiwell Tumor Invasion System (A) and BD BioCoat 24-Multiwell Tumor Invasion System (B) were assayed. Fluorescently labeled cells residing on the bottom of the insert membrane were measured post-invasion with either a Victor2™ plate reader (BD BioCoat 96-Multiwell Tumor Invasion System) or a CytoFluor® plate reader (BD BioCoat 24-Multiwell Tumor Invasion System). Mean percent invasion of NIH-3T3 and HT-1080 cells were compared. Cells were labeled post-invasion using BD Calcein AM.

FIGURE 26 • INHIBITION OF PC3 MIGRATION AND INVASION BY DOXYCYCLINE




PC3 invasion is inhibited by doxycycline. PC3 cell invasion was measured using BD BioCoat 24-Multiwell Tumor Invasion System, which is based on the fluorescence blocking BD FluoroBlok™ PET microporous membrane, and migration was measured using BD Falcon HTS FluoroBlok 24-Multiwell Insert System. At the end of the assay, cells were stained with BD Calcein AM.

# Cell Culture Reagents

## Extracellular Matrix Proteins

| | DESCRIPTION | QTY./CASE | CAT. NO. |
|---|---|---|---|
| BD Matrigel™ Basement Membrane Matrix | BD Matrigel Matrix | 5 ml | 47743-706 |
| | BD Matrigel Matrix | 10 ml | 47743-715 |
| | BD Matrigel Matrix (50 ml) | 5 x 10 ml | 47743-710 |
| | BD Matrigel Matrix High Concentration (HC) | 10 ml | 47747-220 |
| | BD Matrigel Matrix Phenol Red-Free | 10 ml | 47743-716 |
| | BD Matrigel Matrix HC Phenol Red-free | 10 ml | 80094-328 |
| | BD Matrigel Matrix Growth Factor Reduced (GFR) | 5 ml | 47743-718 |
| | BD Matrigel Matrix GFR | 10 ml | 47743-720 |
| | BD Matrigel Matrix HC GFR | 10 ml | 80094-330 |
| | BD Matrigel Matrix Phenol Red-free GFR | 10 ml | 47743-722 |
| Fibronectin | Fibronectin, human | 1 mg | 47743-728 |
| | Fibronectin, human | 5 mg | 47743-654 |
| | Fibronectin, human (25 mg) | 5 x 5 mg | 47743-730 |
| Collagen I | Collagen I, bovine | 30 mg | 47743-676 |
| | Collagen I, human | 0.25 mg | 47743-688 |
| | Collagen I, rat tail | 100 mg | 47743-656 |
| | Collagen I, rat tail (1 g) | 10 x 100 mg | 47743-712 |
| | Collagen I, human recombinant | 250 ug | 12000-612 |
| Laminin | Laminin, mouse | 1 mg | 47743-734 |
| | Ultra-pure Laminin, mouse | 1 mg | 47743-682 |
| | Laminin/Entactin Complex High Concentration | 10.5 mg | 80085-668 |
| Poly-D-Lysine | Poly-D-Lysine, synthetic | 20 mg | 47743-736 |

## Cytokines and Media Additives

| | DESCRIPTION | QTY./CASE | CAT. NO. |
|---|---|---|---|
| Epidermal Growth Factor (EGF) | Mouse, natural (culture grade) | 100 µg | 62405-786 |
| | Mouse, natural (culture grade) (10 x 100 µg) | 1 mg | 47743-756 |
| | Mouse, natural (receptor grade) | 100 µg | 47743-562 |
| | Mouse, natural (receptor grade) (5 x 100 µg) | 500 µg | 47743-564 |
| | Human recombinant | 100 µg | 47743-566 |
| | Human recombinant (10 x100 µg) | 1 mg | 47743-568 |
| Basic Fibroblast Growth Factor (bFGF) | bFGF, bovine natural | 10 µg | 47743-572 |
| | bFGF, human recombinant | 10 µg | 47743-574 |
| | bFGF, human recombinant (50 µg) | 5 x 10 µg | 47743-670 |
| | bFGF, human recombinant (100 µg) | 10 x 10 µg | 47743-672 |
| ITS Universal Culture Supplement Premix | 5 liter equivalent | 5 ml | 47743-626 |
| | 20 liter equivalent | 20 ml | 47743-624 |
| Nerve Growth Factor (NGF) | 2.5S NGF, mouse natural | 10 µg | 47743-588 |
| | 2.5S NGF, mouse natural | 100 µg | 47743-590 |
| | 2.5S NGF, mouse natural (1 mg) | 2 x 500 µg | 47743-592 |
| | 7S NGF, mouse natural | 100 µg | 47743-594 |
| Vascular Endothelial Growth Factor (VEGF) | Human recombinant | 10 µg | 47743-610 |
| MITO+ Serum Extender | 5 liter equivalent | 5 ml | 47743-630 |
| Endothelial Cell Growth Supplement (ECGS) | Bovine | 15 mg | 62405-784 |
| | Bovine | 100 mg | 47743-652 |
| Specialty Media | E-STIM Endothelial Cell Culture Medium | 500 ml | 62405-070 |
| | Hepatocyte Culture Media | 500 ml | 62405-898 |
| | Intestinal Differentiation Media Pack | 1 pack | 62405-442 |
| | Seeding Basal Medium | 2 x 250 ml | 62405-444 |
| | Enterocyte Differentiation Medium | 2 x 250 ml | 62405-446 |
| HUVEC-2 Cells | HUVEC-2 Cells | 1 cryovial | BD354151 |

## BD™ Cell Recovery/Detachment Reagents

| | | | |
|---|---|---|---|
| Cell Recovery Reagents | Dispase | 100 ml | 47743-724 |
| | Cell Recovery Solution | 100 ml | 47743-696 |

## BD Fluorescent Dyes

| | | | |
|---|---|---|---|
| Fluorescent Dyes | Calcein AM Fluorescent Dye | 10 x 50 µg | 89044-502 |
| | Calcein AM Fluorescent Dye | 1 mg | 89044-504 |
| | $DiIC_{12}(3)$ Fluorescent Dye | 100 mg | 89044-506 |

# Cell Culture Tools

## BD BioCoat™ Collagen I Cellware

| DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|
| 6-well plates | 5 | 62405-601 |
| 6-well plates (10 sleeves of 5) | 50 | 62405-413 |
| 12-well plates | 5 | 62405-603 |
| 12-well plates (10 sleeves of 5) | 50 | 62405-415 |
| 24-well plates | 5 | 62405-607 |
| 24-well plates (10 sleeves of 5) | 50 | 62405-417 |
| 48-well plates | 5 | 62405-609 |
| 48-well plates (10 sleeves of 5) | 50 | 62406-020 |
| 96-well plates | 5 | 62405-611 |
| 96-well plates (10 sleeves of 5) | 50 | 62405-419 |
| 96-well plates | 80 | 47748-278 |
| 96-well black/clear plates | 5 | 62406-290 |
| 96-well black/clear plates (10 sleeves of 5) | 50 | 62406-266 |
| 96-well black/clear plates | 80 | 47748-282 |
| 96-well white/clear plates | 5 | 62406-388 |
| 96-well white/clear plates (10 sleeves of 5) | 50 | 62406-386 |
| 96-well white plates | 5 | 62406-288 |
| 96-well white plates (10 sleeves of 5) | 50 | 62406-286 |
| 96-well white plates | 80 | 47748-280 |
| 96-well white/clear plates | 80 | 47748-284 |
| 35 mm culture dishes | 20 | 62405-613 |
| 35 mm culture dishes (5 sleeves of 20) | 100 | 62405-421 |
| 60 mm culture dishes | 20 | 62405-615 |
| 60 mm culture dishes (5 sleeves of 20) | 100 | 62405-423 |
| 100 mm culture dishes | 10 | 62405-617 |
| 100 mm culture dishes (4 sleeves of 10) | 40 | 62405-425 |
| 150 mm culture dishes | 5 | 62405-619 |
| 25 $cm^2$ plug-seal flasks | 10 | 62405-539 |
| 25 $cm^2$ plug-seal flasks (5 sleeves of 10) | 50 | 62405-427 |
| 25 $cm^2$ vented-cap flasks | 10 | 62405-025 |
| 25 $cm^2$ vented-cap flasks (5 sleeves of 10) | 50 | 12777-072 |
| 75 $cm^2$ plug-seal flasks | 5 | 62405-621 |
| 75 $cm^2$ plug-seal flasks (10 sleeves of 5) | 50 | 62405-429 |
| 75 $cm^2$ vented-cap flasks | 5 | 12777-074 |
| 75 $cm^2$ vented-cap flasks (10 sleeves of 5) | 50 | 12777-076 |
| 150 $cm^2$ plug-seal flasks | 5 | 62405-875 |
| 150 $cm^2$ plug-seal flasks (8 sleeves of 5) | 40 | 62406-022 |
| 150 $cm^2$ vented-cap flasks | 5 | 12777-078 |
| 150 $cm^2$ vented-cap flasks (8 sleeves of 5) | 40 | 12777-080 |
| Coverslips 22 mm round No.1 German glass | 60 | BD354089 |
| 1-well CultureSlides | 12 | 62406-704 |
| 2-well CultureSlides | 12 | 62405-876 |
| 4-well CultureSlides | 12 | 62406-716 |
| 8-well CultureSlides | 12 | 62405-878 |

## BD BioCoat™ Poly-D-Lysine Cellware

| DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|
| 6-well plates | 5 | 62405-747 |
| 6-well plates (10 sleeves of 5) | 50 | 62406-024 |
| 12-well plates | 5 | 62406-026 |
| 12-well plates (10 sleeves of 5) | 50 | 62406-028 |
| 24-well plates | 5 | 62405-749 |
| 24-well plates (10 sleeves of 5) | 50 | 62406-030 |
| 48-well plates | 5 | 62406-718 |
| 48-well plates (10 sleeves of 5) | 50 | 62406-700 |
| 96-well plates | 5 | 62405-027 |
| 96-well plates (10 sleeves of 5) | 50 | 62406-032 |
| 96-well plates | 80 | 47748-262 |
| 96-well black/clear plates | 5 | 62406-034 |
| 96-well black/clear plates (10 sleeves of 5) | 50 | 62406-036 |
| 96-well black/clear plates | 80 | 47748-266 |
| 96-well white/clear plates | 5 | 62406-368 |
| 96-well white/clear plates (10 sleeves of 5) | 50 | 62406-366 |
| 96-well white/clear plates | 80 | 47748-268 |
| 96-well white plates | 5 | 62406-284 |
| 96-well white plates (10 sleeves of 5) | 50 | 62406-282 |
| 96-well white/opaque plates | 80 | 47748-264 |
| 35 mm culture dishes | 20 | 62406-038 |
| 35 mm culture dishes (5 sleeves of 20) | 100 | 62406-040 |
| 60 mm culture dishes | 20 | 62406-042 |
| 60 mm culture dishes (5 sleeves of 20) | 100 | 62406-044 |
| 100 mm culture dishes | 10 | 62406-084 |
| 100 mm culture dishes (4 sleeves of 10) | 40 | 62406-050 |
| 150 mm culture dishes | 5 | 62406-702 |
| 25 $cm^2$ plug-seal flasks | 10 | 62405-752 |
| 25 $cm^2$ plug-seal flasks (5 sleeves of 10) | 50 | 62406-052 |
| 25 $cm^2$ vented-cap flasks | 10 | 12777-088 |
| 25 $cm^2$ vented-cap flasks (5 sleeves of 10) | 50 | 12777-090 |
| 75 $cm^2$ plug-seal flasks | 5 | 62405-755 |
| 75 $cm^2$ plug-seal flasks (10 sleeves of 5) | 50 | 62406-054 |
| 75 $cm^2$ vented-cap flasks | 5 | 12777-092 |
| 75 $cm^2$ vented-cap flasks (10 sleeves of 5) | 50 | 12777-094 |
| 150 $cm^2$ plug-seal flasks | 5 | 62405-887 |
| 150 $cm^2$ plug-seal flasks (8 sleeves of 5) | 40 | 62406-056 |
| 150 $cm^2$ vented-cap flasks | 5 | 12777-084 |
| 150 $cm^2$ vented-cap flasks (8 sleeves of 5) | 40 | 12777-096 |
| Coverslips 12 mm round No.1 German glass | 80 | BD354086 |
| 35 mm Coverslip-bottom dishes | 20 | BD354077 |
| 1-well CultureSlides | 12 | 62406-709 |
| 2-well CultureSlides | 12 | 62405-888 |
| 4-well CultureSlides | 12 | 62406-714 |
| 8-well CultureSlides | 12 | 62405-890 |

## BD BioCoat™ Poly-L-Lysine Cellware

| DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|
| 6-well plates | 5 | 62406-280 |
| 6-well plates (10 sleeves of 5) | 50 | 62406-278 |
| 96-well plates | 5 | 62406-276 |
| 96-well plates (10 sleeves of 5) | 50 | 62406-274 |
| 35 mm culture dishes | 20 | 62406-272 |
| 35 mm culture dishes (5 sleeves of 20) | 100 | 62406-270 |
| 60 mm culture dishes | 20 | 62406-268 |
| 60 mm culture dishes (5 sleeves of 20) | 100 | 62406-292 |
| Coverslips 12 mm round No.1 German glass | 80 | BD354085 |

## BD BioCoat™ Laminin Cellware

| DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|
| 6-well plates | 5 | 62405-694 |
| 12-well plates | 5 | 62405-696 |
| 24-well plates | 5 | 62405-698 |
| 48-well plates | 5 | 62405-700 |
| 96-well plates | 5 | 62405-702 |
| 35 mm culture dishes | 20 | 62405-704 |
| 60 mm culture dishes | 20 | 62405-706 |
| 100 mm culture dishes | 10 | 62405-708 |
| 150 mm culture dishes | 5 | 62405-710 |
| 25 $cm^2$ plug-seal flasks | 10 | 62405-048 |
| 75 $cm^2$ plug-seal flasks | 10 | 62405-712 |

## BD BioCoat™ Matrigel™ Matrix – for Hepatocytes

| DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|
| 6-well plates | 5 | 62405-144 |

## BD BioCoat™ Matrigel™ Matrix Plates for Embryonic Stem Cell Culture

| DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|
| 6-well plates | 5 | 89022-350 |

## BD BioCoat™ Poly-D-Lysine/Laminin Cellware

| DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|
| 6-well plates | 5 | 62405-109 |
| 24-well plates | 5 | 62405-111 |
| 96-well plates | 5 | 62405-113 |
| 100 mm culture dishes | 10 | 62405-115 |
| Coverslips 12 mm round No.1 German glass | 80 | BD354807 |
| 2-well CultureSlides | 12 | BD354687 |
| 8-well CultureSlides | 12 | BD354688 |

## BD BioCoat™ Poly-L-Ornithine/Laminin Cellware

| DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|
| 6-well plates | 5 | 62405-434 |
| 24-well plates | 5 | 62405-436 |
| 96-well plates | 5 | 62405-438 |

## BD Falcon™ Cultureware

| | DESCRIPTION | QTY./CASE | CAT. NO. |
|---|---|---|---|
| 1-well CultureSlides | 8.6 $cm^2$ growth surface area per well | 96 | 62405-172 |
| | | 24 | 53106-300 |
| 2-well CultureSlides | 4.0 $cm^2$ growth surface area per well | 96 | 62405-174 |
| | | 24 | 53106-302 |
| 4-well CultureSlides | 1.7 $cm^2$ growth surface area per well | 96 | 62405-176 |
| | | 24 | 53106-304 |
| 8-well CultureSlides | 0.7 $cm^2$ growth surface area per well | 96 | 62405-178 |
| | | 24 | 53106-306 |
| BD Primaria™ Cell Culture Dishes with lid | 35x10 mm style | 200 | 25382-654 |
| | 60x15 mm style | 200 | 25382-687 |
| | 100x20 mm style | 200 | 25382-701 |
| BD Primaria™ Cell Culture Flasks with plug-seal screw cap | 25 $cm^2$ growth area, 50 ml, canted neck | 200 | 29184-801 |
| | 75 $cm^2$ growth area, 250 ml, straight neck | 100 | 29184-845 |
| BD Primaria™ Cell Culture Flasks with 0.2 µm membrane vented screw cap | 25 $cm^2$ growth area, 50 ml, canted neck | 100 | 15705-068 |
| | 75 $cm^2$ growth area, 250 ml, straight neck | 100 | 15705-070 |
| BD Primaria™ Cell Culture Plates, flat-bottom with lid | 6-well | 50 | 62406-455 |
| | 24-well | 50 | 62406-456 |
| | 96-well | 50 | 62406-503 |
| 96-well Imaging Plate | Black/Clear, with lid | 32 | 89022-352 |

## Cell Environments

| | DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|---|
| BD BioCoat™ Tumor Invasion System | One insert plate with one 24-well plate and lid | 1 | 47733-148 |
| | Five insert plates with five 24-well plates and lids | 5 | 47733-146 |
| BD BioCoat Angiogenesis System: Endothelial Cell Invasion | One insert plate with one 24-well plate and lid | 1 | BD354141 |
| | Five insert plates with five 24-well plates and lids | 5 | BD354142 |
| BD BioCoat Angiogenesis System: Endothelial Cell Migration | One insert plate with one 24-well plate and lid | 1 | 47743-788 |
| | Five insert plates with five 24-well plates and lids | 5 | 47743-790 |
| | One insert plate with one 96-well plate and lid | 1 | 47747-300 |
| | Five insert plates with five 96-well plates and lids | 5 | 47747-302 |
| BD BioCoat Angiogenesis System: Endothelial Tube Formation | 96-well Black/Clear BD Optilux™ Microplate | 1 | 47745-226 |
| | 96-well Black/Clear Optilux Microplate | 5 | 47745-228 |
| BD BioCoat BD Matrigel™ Invasion Chambers | 8 µm inserts in four 6-well plates | 24 | 62405-742 |
| | 8 µm inserts in two 24-well plates | 24 | 62405-744 |
| BD BioCoat GFR Matrigel Invasion Chambers | 8 µm inserts in two 24-well plates | 24 | 62405-158 |
| BD BioCoat Endothelial Cells | Endothelial Cell Growth Environment | 1 | 62405-590 |

| | DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|---|
| BD BioCoat Hepatocyte Differentiation | Hepatocyte Differentiation Environment | 1 | 62405-096 |
| BD BioCoat Intestinal Epithelial Differentiation Environment | Intestinal Epithelium Differentiation Environment | 1 | 62405-098 |
| BD BioCoat HTS Caco-2 Assay Systems | 1 µm inserts in one 24-Multiwell plate with feeder tray and lid | 1 | 12777-040 |
| | 1 µm inserts in one 24-Multiwell plate with feeder tray and lid | 5 | 12777-042 |
| BD BioCoat Fibrillar Collagen 24-Multiwell Insert Systems | 1 µm inserts in one 24-Multiwell plate with feeder tray and lid | 1 | 12777-044 |
| | 1 µm inserts in one 24-Multiwell plate with feeder tray and lid | 5 | 12777-046 |

## Membrane Insert Systems

*For use with BD Falcon™ Cell Culture Insert Companion Plates*

| | DESCRIPTION | QTY./CASE | CAT. NO. |
|---|---|---|---|
| 0.4 µm, Transparent PET membrane | for 6-well plates | 48 | 62406-163 |
| | for 12-well plates | 48 | 62406-174 |
| | for 24-well plates | 48 | 62404-550 |
| 1 µm, Transparent PET membrane | for 6-well plates | 48 | 62406-171 |
| | for 12-well plates | 48 | 62406-172 |
| | for 24-well plates | 48 | 62406-173 |
| 3 µm, Transparent PET membrane | for 6-well plates | 48 | 62406-164 |
| | for 12-well plates | 48 | 62406-174 |
| | for 24-well plates | 48 | 62406-169 |
| 0.4 µm, HD inserts Translucent PET membrane | for 6-well plates | 48 | 62406-179 |
| | for 12-well plates | 48 | 62406-180 |
| | for 24-well plates | 48 | 62406-181 |
| 3 µm HD Inserts, Translucent PET membrane | for 6-well plates | 48 | 62406-170 |
| | for 12-well plates | 48 | 62406-177 |
| | for 24-well plates | 48 | 62406-178 |
| 8 µm Translucent PET membrane | for 6-well plates | 48 | 62406-196 |
| | for 12-well plates | 48 | 62406-176 |
| | for 24-well plates | 48 | 62406-198 |
| BD Falcon Cell Culture Insert Companion Plates | 6-well plate | 50 | 62406-185 |
| | 12-well plate | 50 | 62406-187 |
| | 24-well plate | 50 | 62406-189 |
| BD Falcon 24-Multiwell Insert Systems | 1 µm PET membrane | 1 | 62406-222 |
| | 1 µm PET membrane | 5 | 47727-004 |
| | 3 µm PET membrane | 1 | 62406-226 |
| | 3 µm PET membrane | 5 | BD351183 |
| | 8 µm PET membrane | 1 | 62406-230 |
| | 8 µm PET membrane | 5 | BD351185 |
| BD Falcon 24-Multiwell Insert Systems | Feeder tray with lid | 5 | BD351186 |
| BD Falcon FluoroBlok™ 96-Multiwell Insert Systems | 3 µm, One insert plate with 96-well plate and lid | 1 | BD351161 |
| | 8 µm, Five insert plates with 96-well plates and lids | 5 | BD351164 |
| BD Falcon 96-Square Well, Flat-Bottom Plate and Lid | 96-square well, flat-bottom plate and lid | 5 | BD353928 |

| | DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|---|
| BD BioCoat™ Collagen I Cell Culture Inserts | 0.4 µm inserts in four 6-well plates | 24 | 62405-626 |
| | 0.4 µm inserts in four 12-well plates | 24 | 62405-540 |
| | 0.4 µm inserts in two 24-well plates | 24 | 62405-628 |
| | 1 µm inserts in four 6-well plates | 24 | 62405-542 |
| | 1 µm inserts in four 12-well plates | 24 | 62405-544 |
| | 1 µm inserts in two 24-well plates | 24 | 62405-546 |
| | 3 µm inserts in four 6-well plates | 24 | 62405-630 |
| | 3 µm inserts in four 12-well plates | 24 | 62405-634 |
| | 3 µm inserts in two 24-well plates | 24 | 62405-632 |
| BD BioCoat Collagen IV Cell Culture Inserts | 1 µm inserts in two 24-well plates | 24 | 62405-554 |
| | 3 µm inserts in four 6-well plates | 24 | 62405-654 |
| | 3 µm inserts in two 24-well plates | 24 | 62405-656 |
| BD BioCoat Fibrillar Collagen Cell Culture Inserts | 1 µm inserts in four 6-well plates | 24 | 62405-119 |
| | 1 µm inserts in four 12-well plates | 24 | 62405-121 |
| | 1 µm inserts in two 24-well plates | 24 | 62405-050 |
| BD BioCoat Fibronectin Cell Culture Inserts | 0.4 µm inserts in four 6-well plates | 24 | 62405-686 |
| | 0.4 µm inserts in two 24-well plates | 24 | 62405-688 |
| | 1 µm inserts in two 24-well plates | 24 | 62405-566 |
| | 3 µm inserts in four 12-well plates | 24 | 62405-568 |
| | 3 µm inserts in two 24-well plates | 24 | 62405-655 |
| BD BioCoat FluoroBlok Fibronectin Cell Culture Inserts | 3 µm inserts in two 24-well plates | 24 | BD354597 |
| BD BioCoat Collagen I 24-Multiwell Insert System | 3 µm insert plate with 24-well plate and lid | 1 | BD354598 |
| BD BioCoat™ Fibronectin 24-Multiwell Insert System | 3 µm insert plate with 24-well plate and lid | 1 | BD354599 |

## Membrane Insert Systems (continued)

| | DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|---|
| BD BioCoat™ Collagen I Cell Culture Inserts | 0.4 µm inserts in four 6-well plates | 24 | 62405-626 |
| | 0.4 µm inserts in four 12-well plates | 24 | 62405-540 |
| | 0.4 µm inserts in two 24-well plates | 24 | 62405-628 |
| | 1 µm inserts in four 6-well plates | 24 | 62405-542 |
| | 1 µm inserts in four 12-well plates | 24 | 62405-544 |
| | 1 µm inserts in two 24-well plates | 24 | 62405-546 |
| | 3 µm inserts in four 6-well plates | 24 | 62405-630 |
| | 3 µm inserts in four 12-well plates | 24 | 62405-634 |
| | 3 µm inserts in two 24-well plates | 24 | 62405-632 |
| BD BioCoat Collagen IV Cell Culture Inserts | 1 µm inserts in two 24-well plates | 24 | 62405-554 |
| | 3 µm inserts in four 6-well plates | 24 | 62405-654 |
| | 3 µm inserts in two 24-well plates | 24 | 62405-656 |
| BD BioCoat Fibrillar Collagen Cell Culture Inserts | 1 µm inserts in four 6-well plates | 24 | 62405-119 |
| | 1 µm inserts in four 12-well plates | 24 | 62405-121 |
| | 1 µm inserts in two 24-well plates | 24 | 62405-050 |
| BD BioCoat Fibronectin Cell Culture Inserts | 0.4 µm inserts in four 6-well plates | 24 | 62405-686 |
| BD BioCoat FluoroBlok™ Fibronectin Cell Culture Inserts | 3.0 µm inserts in two 24-well plates | 24 | BD354597 |
| BD BioCoat Collagen I 24-Multiwell Insert System | 3.0 µm insert plate with 24-well plate and lid | 1 | BD354598 |
| BD BioCoat Fibronectin 24-Multiwell Insert System | 3.0 µm insert plate with 24-well plate and lid | 1 | BD354599 |
| BD BioCoat Laminin Cell Culture Inserts | 0.4 µm inserts in two 24-well plates | 24 | 62405-718 |
| | 3 µm inserts in two 24-well plates | 24 | 62405-722 |

| | DESCRIPTION | QTY./CASE | CAT. NO |
|---|---|---|---|
| BD BioCoat Matrigel™ Matrix Cell Culture Inserts | 0.4 µm inserts in four 6-well plates | 24 | 62405-734 |
| | 0.4 µm inserts in two 24-well plates | 24 | 62405-736 |
| BD BioCoat Control Cell Culture Inserts | 0.4 µm inserts in four 6-well plates | 24 | 62405-020 |
| | 0.4 µm inserts in four 12-well plates | 24 | 62405-018 |
| | 0.4 µm inserts in two 24-well plates | 24 | 62405-016 |
| | 1 µm inserts in four 6-well plates | 24 | 62405-014 |
| | 1 µm inserts in four 12-well plates | 24 | 62405-012 |
| | 1 µm inserts in two 24-well plates | 24 | 62405-010 |
| | 3 µm inserts in four 6-well plates | 24 | 62405-008 |
| | 3 µm inserts in four 12-well plates | 24 | 62405-006 |
| | 3 µm inserts in two 24-well plates | 24 | 62405-004 |
| | 8 µm inserts in four 6-well plates | 24 | 62405-002 |
| | 8 µm inserts in two 24-well plates | 24 | 62405-000 |
| BD Falcon™ FluoroBlok Cell Culture Inserts<br>For use with BD Falcon 24-well Cell Culture Insert Companion Plates (Cat. No. 353504) | 1 µm inserts | 48 | 62406-498 |
| | 3 µm inserts | 48 | 62406-500 |
| | 8 µm inserts | 48 | 62406-504 |
| | 1 µm insert system in one 24-well plate | 5 | BD351154 |
| | 3 µm insert system in one 24-well plate | 1 | BD351155 |
| | 3 µm insert system in one 24-well plate | 5 | BD351156 |
| | 8 µm insert system in one 24-well plate | 1 | BD351157 |
| | 8 µm insert system in one 24-well plate | 5 | BD351158 |
| BD BioCoat Deep-Well Plates<br>For use with BD BioCoat Cell Culture Inserts | 6-well Deep-Well Plates | 4 | 62405-150 |

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