

Cell Growth and Differentiation







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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 Corning offers a wide range of extracellular matrix proteins and attachment factors for researchers to incorporate into their cell culture systems. For over 20 years, we have 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: Corning[®] BioCoat[™] Cellware. Our extensive experience in protein purification, along with rigorous quality assurance testing guarantees high-quality, consistent products.

At Corning 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. Falcon[®], Corning BioCoat, and Corning 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 threedimensional 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 Corning 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

Corning offers a wide variety of surface chemistries and attachment factors appropriate for a broad range of applications. The surface of our 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. Corning[®] Primaria[™] and Corning 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. Corning PureCoat[™] surfaces are a novel family of chemically synthesized and animal-free surfaces that enhance cell attachment and growth in low-serum or serum-free culture environments. 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.

Falcon Non-treated Polystyrene

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

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.

Corning 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).

Corning 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.

Corning 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.

Corning 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.

Corning 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.

Corning 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.

Corning 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.

Corning 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.

Corning 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.

Corning 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.

Corning 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.

Corning PureCoat ECM Mimetic Fibronectin Peptide

- Consists of RGD sequences to support the attachment of cell types that require Fibronectin coating including alpha-5 integrin-positive cells.
- Compatible, animal-free alternative to natural animal or human ECM surfaces, such as natural human Fibronectin for hMSC expansion and differentiation.

Corning PureCoat ECM Mimetic Collagen I Peptide

- Supports the attachment of Collagen I-dependent cell types including alpha 2 integrin-positive cells (and others).
- Compatible, animal-free alternative to natural animal or human ECM surfaces, such as natural human Collagen I for human keratinocyte expansion.

PRODUCT SELECTION BY CELL TYPE

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Corning BioCoat Fibrillar Collagen Cell Culture Inserts								Corning BioCoat Tumor Invasion System	
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For guideline use only. This is not a complete list of all applications for these products.



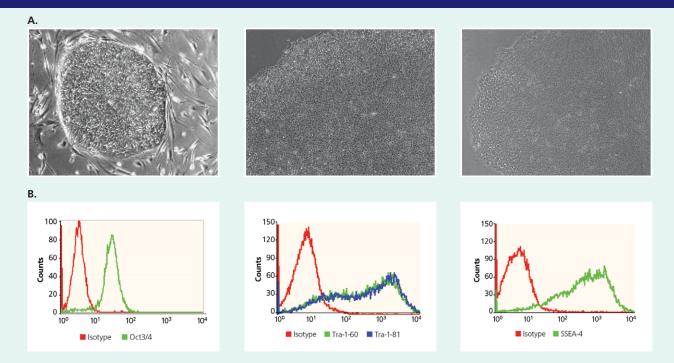
Human Embryonic Stem Cells

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. Corning® Matrigel® Matrix, coupled with a variety of culture media, has been widely accepted as an alternative substrate to feeder-dependent culture of hES cells¹⁻⁴, and Corning Matrigel Matrix has been used to culture iPS cells⁵⁻⁶. Corning Matrigel Matrix is a reconstituted basement membrane isolated from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma.

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

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

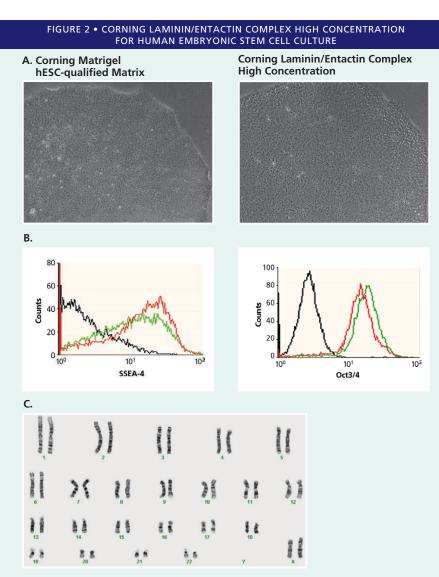


1A. Phase contrast images of H9 colonies grown on mouse embryonic fibroblast (MEF) feeder layer in hES media (left), Corning Matrigel hESC-qualified Matrix in MEF-conditioned media (middle), or mTeSR®1 maintenance media (right). Images were taken at 4x magnification.

1B. Flow cytometry analysis of H9 cells cultured on Corning 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 BD CellQuest[™] software.

maintain and expand hES cells in an undifferentiated state when used with Corning Matrigel[®] hESC-qualified Matrix as a substrate (**Figure 1**).

An alternative surface for hES cell culture is Corning Laminin/Entactin Complex High Concentration (**Figure 2**). Corning 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 Corning Matrigel hESC-qualified Matrix, this surface is not specifically qualified for maintenance of undifferentiated hES cells.



2A. Phase contrast images of H9 cells grown on Corning Matrigel hESC-qualified Matrix (left) and Corning 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 Corning Laminin/Entactin Complex High Concentration (red line) and Corning 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 BD CellQuest™ software. Both surfaces supported undifferentiated expansion of hESC, H9.

2C. G banding chromosome analysis. Karyotype analysis of H9 cells grown on Corning 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 Cul	ture Reagents	
Extracell	ular Matrix Proteins	
354277	Corning Matrigel hESC-qualified Matrix	5 mL
354259	Laminin/Entactin Complex High Concentration	10.5 mg
Cytokine	es and Media Addtives	
354060	bFGF, human recombinant	10 µg
Cell Reco	overy Reagents	
354235	Dispase	100 mL
354253	Cell Recovery Solution	100 mL
Cell Cul	ture Tools	
	BioCoat™ Matrigel Matrix yonic Stem Cell Culture	Plates
354671	6-well Plates	5
Falcon [®] N	Aultiwell Cell Culture Plates	
353046	6-well Flat-bottom with lid,	1

For a complete product listing, see page 19.

Tissue Culture-treated

DID YOU KNOW?

• Corning offers a full range of pipets and tubes. Please contact your sales representative for more information.

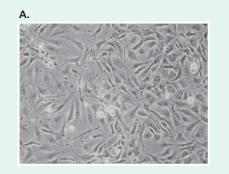


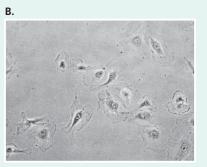
Endothelial Cells

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 migration⁷, invasion⁸, and tubule formation⁹⁻¹⁵. Both the Corning[®] BioCoat[™] Angiogenesis System: Endothelial Cell Invasion and the Corning BioCoat Angiogenesis System: Endothelial Cell Migration allow for rapid data collection without multiple handling steps. These quantitative assays utilize Corning 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 Corning® BioCoat Angiogenesis System: Endothelial Cell Invasion are evenly coated with Corning 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 CORNING BIOCOAT ENDOTHELIAL CELL GROWTH ENVIRONMENT ON HUVEC



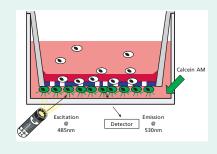


Corning BioCoat Endothelial Cell Growth Environment utilizes Corning BioCoat Collagen I Cellware and Corning Endothelial Cell Culture Medium to enhance endothelial attachment and proliferation. HUVECs grown for five days using the Corning BioCoat Endothelial Cell Growth Environment form a confluent monolayer 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).



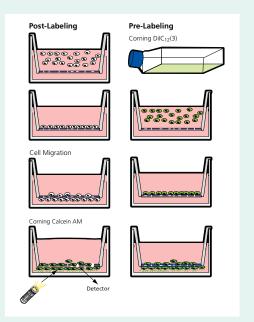
• The use of Corning Cell Recovery Solution or Corning Dispase is necessary to recover cells cultured on Corning Matrigel Matrix.

FIGURE 4 • LABELING CELLS POST-INVASION WITH CALCEIN AM



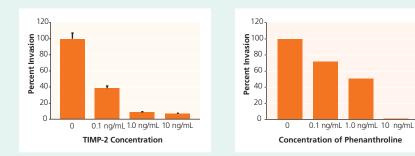
A fluorescence plate reader quantifies cells post-invasion by measuring fluorescence which correlates to cell number. Cells on top of the Corning[®] FluoroBlok[™] membrane are not detected by a bottom-reading fluorometer.

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



Corning 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 Corning 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 Corning $DilC_{12}(3)$. 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 Corning BioCoat[™] Angiogenesis System: Endothelial Cell Invasion in the presence of VEGF (4 μ g/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 Corning Calcein AM (4 μ g/mL) and then analyzed for invasion through Corning 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 Cu	lture Reagents	

Extracellular Matrix Proteins

Exclusion		
354230	Corning Matrigel	10 mL
	Basement Membrane Matrix	
	Growth Factor Reduced	

Cell Recovery Reagents

354235	Dispase	100 mL
354253	Cell Recovery Solution	100 mL

Fluorescent Dyes

354218	DilC ₁₂ (3)	100 mg
354216	Calcein AM	10 x 50 µg

HUVEC Cells				
354151	HUVEC-2 Cells	1 cryovial		
Specialty	/ Media			
355054	Endothelial Cell	500 mL		

Culture Media Cytokines and Media Additives

354006	Endothelial Cell Growth Supplement, bovine	15 mg
354107	Vascular Endothelial Growth Factor, human recombinant	10 µg

Cell Culure Tools

Corning BioCoat Collagen I Cellware

354450 100 mm Dish 10

Cell Environments

Corning BioCoat Cell Environment

355053	Endothelial Cell	1
	Growth Environment	

Membrane Insert Systems

Corning BioCoat Angiogenesis System: Endothelial Cell Migration

354143 24-Multiwell Insert Plate with lid 1

Corning BioCoat Angiogenesis System: Endothelial Cell Invasion

354141 24-Multiwell Insert Plate with lid 1

Corning BioCoat Angiogenesis System: Endothelial Tube Formation

354149 96-Multiwell Insert Plate with lid 1

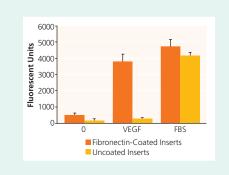
For a complete product listing, see page 19.

Corning[®] BioCoat[™] Angiogenesis System: Endothelial Cell Migration consists of Corning FluoroBlok[™] inserts evenly coated with human fibronectin (**Figure 7**). Studies conducted using the post-labeling technique demonstrated that Corning 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*.

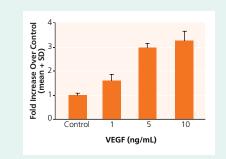
* Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation offers a standardized and robust assay for studying endothelial cell tubulogenesis. For customers interested in establishing an assay for tube formation using vialed Corning Matrigel® Matrix, we recommend pre-testing lots to ensure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

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



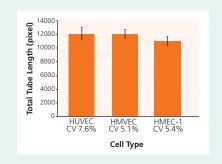
Migration assays were conducted using HUVECs in the Corning BioCoat Angiogenesis System: Endothelial Cell Migration and compared with uncoated Corning FluoroBlok 24-Multiwell Inserts using both FBS (5%) and VEGF (10 µg/ mL) as chemoattractants. The cells were allowed to migrate for 22 \pm 1 hour. Cells were labeled post-migration with Calcein AM (4 µg/mL) and measured by detecting the fluorescence of the cells that migrated through the Corning 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 \pm S.D.

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



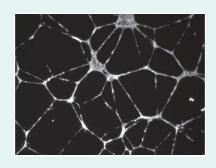
Corning HUVEC-2 cells assayed in the Corning BioCoat Angiogenesis System: Endothelial Cell Migration (96-Multiwell format) in response to increasing concentrations of VEGF. Samples were incubated for 22 hours. Cells were labeled post-migration with Corning Calcein AM and measured by detecting the fluorescence of cells that migrated through the fibronectin-coated Corning 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 Corning[®] BioCoat[™] Angiogenesis System: Endothelial Cell Tube Formation (**Figures 9-11**) which is comprised of a 3D gel of Corning Matrigel[®] Matrix. The Corning BioCoat Angiogenesis Systems are available in 24-and 96-Multiwell formats, which can be used for moderate to high throughput compound screening. Corning 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



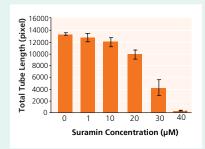
HUVEC, HMVEC, and the human endothelial cell line HMEC-1 exhibit tube formation on Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation. For this study, 20,000 cells of each cell type were added to wells containing presolidified Corning Matrigel Matrix. The assay was incubated for 18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

FIGURE 10 • CONFOCAL IMAGE OF CORNING HUVEC-2 CELL TUBE FORMATION



Corning HUVEC-2 cells were assayed using the Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation. Cells were stained using Corning 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 Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation. 50 µl of cells plus compound were added to wells containing presolidified Corning Matrigel Matrix. Samples were incubated at 37°C, 5% CO₂ for 18 hours before staining with Corning 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.

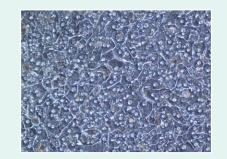
Pseudo-colored image for illustrative purposes only.

Hepatocytes

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 I19-22, Corning® Matrigel® Matrix23-27 or Corning PuraMatrix^{™28-29}. Corning BioCoat[™] Collagen I Cellware is a commonly used surface for cultures of both fresh and cryopreserved hepatocytes³⁰⁻³¹ (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 Corning BioCoat Collagen I with Corning Matrigel Matrix overlay, are used to assess bile canaliculi formation³². Choly-lysyl-fluorescein (CLF) is a fluorescein-labeled bile acid that is secreted into bile canaliculi by ABC efflux transporters which can be used to visualize bile canaliculi (Figure 14). Corning Matrigel Matrix has been shown to suppress cell growth and prevent growth-associated dedifferentiation²³, as well as maintain liverspecific functions *in vitro* longer than most collagen-based systems²⁴⁻²⁶. Hepatocytes cultured on Corning Matrigel Matrix also have a more differentiated morphology than hepatocytes cultured on collagen I (Figure 15). Both Corning Collagen I and Corning Matrigel Matrix are animal-derived products; Corning 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 • CORNING INDUCIBLE CRYOPRESERVED HUMAN HEPATOCYTES CULTURED ON CORNING BIOCOAT COLLAGEN I

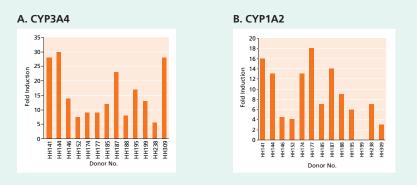


Corning Gentest[™] Inducible-qualified Human CryoHepatocytes were isolated using the Corning Gentest CryoHepatocyte Purification Kit and resuspended in freshly prepared ISOMs seeding media at a concentration of 1x10⁶ cells/ mL. Cells were plated onto Corning BioCoat Collagen I 24-well plates and incubated for approxiamately 2 hours, after which plating media was removed and replaced with supplemented Corning Hepatocyte Culture Media.



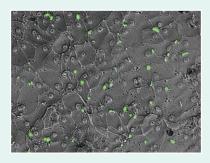
 Corning offers a custom barcoding service. This service provides highquality barcode labels affixed to any side of a microplate.

FIGURE 13 ● INDUCTION OF CORNING GENTEST[™] INDUCIBLE-QUALIFIED HUMAN CRYOHEPATOCYTES



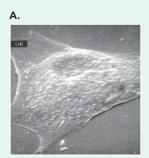
Corning Gentest Inducible-qualified Human CryoHepatocytes were isolated using the Corning Gentest CryoHepatocyte Purification Kit and resuspended into freshly prepared ISOMs seeding media at a concentration of 1x10⁶ cells/mL. Cells were plated onto Corning BioCoat[™] Collagen I 24-well Multiwell Plates and incubated for approximately 2 hours, after which plating media was removed and replaced with supplemented Corning 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.

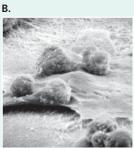
FIGURE 14 • CORNING GENTEST CHOLY-LYSYL-FLUORESCEIN SEQUESTERED IN BILE CANALICULI



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

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





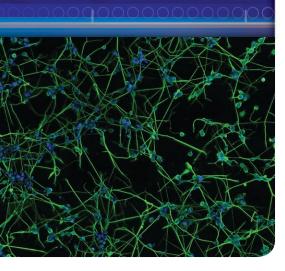


Scanning electron micrographs of primary rat hepatocytes cultured for two days on Collagen I (A), Collagen I gel (B), or Corning Matrigel Matrix (C). Note the clusters of spherical cells for hepatocytes cultured on Corning Matrigel Matrix, typical of differentiated cells.

Tools for Hepatocyte Cell Culture

Cat. No.	Description	Qty
Cell Cul	ture Reagents	
Hepatoc	yte Culture Media Kit	
355056	Maintenance Media	500 m
Extracell	ular Matrix Proteins	
354236	Collagen I, rat tail	100 m
356237	Corning Matrigel [®] Matrix,	10 m
354250	phenol red-free Corning PuraMatrix™	5 m
	Peptide Hydrogel	
	es and Media Additives	
354251	ITS Premix	5 m
Cell Reco	overy Reagents	
354235	Dispase	100 m
354253	Cell Recovery Solution	100 m
Cell Cul	ture Tools	
Cornina	BioCoat™ Collagen I	
354400	6-well plates	
	· · · · · · · · · · · · · · · · · · ·	
	BioCoat Matrigel Culturev	
354510	6-well plates	
Hepato	cytes and Reagents	
Fresh Hu	iman Hepatocytes	
454415	5 million cells per 25 cm² Collagen I Flask	25 cm
454424	24-well plate on BioCoat Collagen I	1 plat
454482	24-well plate on	1 plat
15 1 162	BioCoat Collagen I	i piar
	with Matrigel Overlay	
Inducible	e Human CryoHepatocytes	;
454551	>5 million cells per vial	1.5 m
454550	2-5 million cells per vial	1.5 m
Transpo	rter Human CryoHepatocy	tes
454541	>5million cells per vial	1.5 m
Metabol	ism Human CryoHepatocy	tes
454543	>5 million cells per vial	1.5 m
Cholyl-ly	syl-Fluorescein (CLF)	
451041	Hepatocyte Bile Acid	1 m
451041	Transporter Uptake	1 111
Corning	Gentest [™] Cryopreserved	
Hepatoc	yte Purification Kit	
454500	Purification Kit	1 k
	Purification Kit, One-Step	1 ki
454600		
454600 Gentest	Cryopreserved Hepatocyte	e
454600 Gentest Purificat	Cryopreserved Hepatocyte ion and Plating Medium	
454600 Gentest	Cryopreserved Hepatocyte ion and Plating Medium Recovery and Plating	e 1 ki
454600 Gentest Purificat	Cryopreserved Hepatocyte ion and Plating Medium Recovery and Plating Medium Kit	
454600 Gentest Purificat 454534	Cryopreserved Hepatocyte ion and Plating Medium Recovery and Plating	1 ki
454600 Gentest Purificat 454534 454560 454561	Cryopreserved Hepatocyte ion and Plating Medium Recovery and Plating Medium Kit Recovery Medium Plating Medium	1 ki 45 m
454600 Gentest Purificat 454534 454560 454561	Cryopreserved Hepatocyte ion and Plating Medium Recovery and Plating Medium Kit Recovery Medium	1 ki 45 m
454600 Gentest Purificat 454534 454560 454561 Cell Env	Cryopreserved Hepatocyte ion and Plating Medium Recovery and Plating Medium Kit Recovery Medium Plating Medium	1 ki 45 m 45 m

For a complete product listing, see page 19.

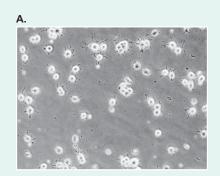


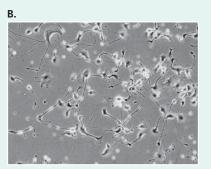
Neuronal Cells

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 Corning[®] 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 Corning BioCoat Poly-D-Lysine Cellware³³ and Corning BioCoat Poly-D-Lysine/Laminin³⁴, 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 Corning BioCoat Poly-L-Lysine Cellware³⁵ and Corning BioCoat Poly-D-Lysine/Laminin Cellware³⁶, respectively. Primary human neural stem cells have been grown under serum-containing conditions in tissue culture-treated Corning Falcon® Cell Culture Flasks³⁷. 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 Corning PuraMatrix[™] while differentiation of neuronal stem cells grown on Corning Matrigel® Matrix was skewed toward GFAP+ astroglial cells³⁸. Both Corning PuraMatrix³⁸⁻⁴⁰ and Corning Primaria^{™41} are defined, xeno-free surfaces for 3D and 2D culture, respectively, which are compatible with neuronal cells. Corning 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 CORNING BIOCOAT LAMININ CELLWARE ON NG-108 NEUROBLASTOMA CELLS



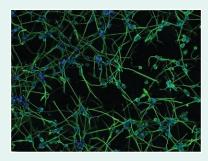


NG-108 rat glioma/mouse neuroblastoma cell morphology is surface dependent. Cells cultured on tissue culture plastic are loosely adhered and remain rounded (A). Cells cultured on Corning BioCoat Laminin cellware exhibit a spindle-shaped morphology and dendritic processes (B).



 Corning 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 CORNING® COLLAGEN I

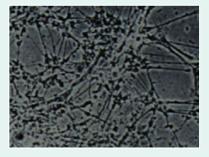


PC12 cells were maintained in DMEM with 10% FBS, 5% horse serum and 1% penicillin/ streptomycin. For neurite generation, approximately 15,000 cells/well were plated in Falcon® 96-well plates that were coated with Corning Collagen I, rat tail using 1.8 µg collagen per well. After 24 hours, the medium 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 μ g/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 CORNING PRIMARIA™ CULTUREWARE

B.





When chick embryo spinal cord neurons are cultured on Corning 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 Corning Primaria Cultureware (B).

Tools for Neuronal Cell Culture

Cat. No.	Description	Qty.
Cell Cu	ulture Reagents	

Extracellular Matrix Proteins

Extracell	ular Matrix Proteins			
354236	Collagen I, rat tail	100 mg		
354008	Fibronectin, human	1 mg		
354232	Laminin, mouse	1 mg		
354234	Corning Matrigel [®] Matrix	10 mL		
354210	Poly-D-Lysine	20 mg		
354250	Corning PuraMatrix™ Peptide Hydrogel	5 mL		
Cytokine	s and Media Additives			
354009	7S Nerve Growth Factor, mouse, natural	100 µg		
354005	2.5S Nerve Growth Factor, mouse, natural	10 µg		
354052	Endothelial Growth Factor, human recombinant	100 µg		
Cell Recovery Reagents				
354235	Dispase	100 mL		
354253	Cell Recovery Solution	100 mL		
C. II. C. II	terme The sile			

Cell Culture Tools

Corning	BioCoat [™] Laminin Cellware	
354404	6-well plates	5
	BioCoat Poly-L-Ornithine/ Cellware	
354657	96-well plates	5
	BioCoat Poly-D-Lysine/ Cellware	
354619	24-well plates	5
Corning	BioCoat Poly-D-Lysine Cellv	vare
354413	6-well	5
Corning	Primaria Cultureware	
353802	60 x 15 mm Dish with lid	200
Falcon™	CultureSlides	
354108	8-well	96
Falcon 9	6-well Plate	
353219	Black/Clear, with lid	32

For a complete product listing, see page 19.

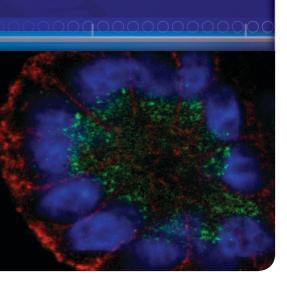


FIGURE 19 • PROLIFERATION OF HUMAN NEONATAL KERATINOCYTES ON CORNING BIOCOAT™ COLLAGEN I



Human neonatal keratinocytes cultured on Corning BioCoat Collagen I.



 Corning offers custom coatings. Please contact your sales representative for more information.

Epithelial Cells

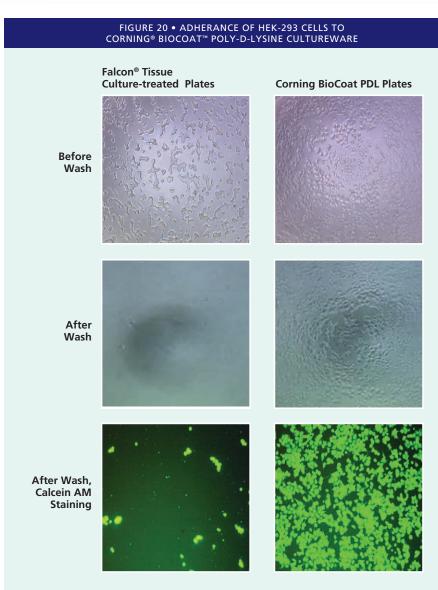
Epithelial cells are found throughout the body, from skin to glandular formations within tissues. *In vivo* these cells are attached to a three dimensional basement membrane matrix. The interactions between the epithelial cell and matrix proteins effect cell morphology and function. Two highly specified 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 Corning[®] BioCoat[™] Cellware provides a range of 2D surfaces for cell growth. Both keratinocytes⁴⁵⁻⁴⁶ and HEK-293⁴⁷⁻⁴⁹ cells are examples of epithelial cells that can be studied in 2D culture environments. Keratinocytes are a major component of the epidermis; Corning 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.

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 epithelial⁵⁰⁻⁵⁴ and Caco-2⁵⁵⁻⁵⁶ 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 Corning Matrigel® Matrix) they form large disorganized colonies, as shown with the T4-vector control in a study from Dr. Bissell's laboratory⁵¹ (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 Corning 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 α 6-integrin (basal marker), β -catenin (basolateral marker) and GM130 (apical marker). These data show that three-dimensional Corning 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 Corning 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. Collagen BioCoat HTS Caco-2 Assay System and Corning BioCoat Intestinal Epithelium Differentiation Environment utilize Collagen BioCoat Fibrilliar Collagen Inserts and a specialized media to enhance the rate of Caco-2 differentiation 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 Corning provide multiple options to researchers studying epithelial cells *in vitro*.



HEK-293 cells have enhanced attachment to Corning BioCoat Poly-D-Lysine Cultureware as compared to Corning Falcon Tissue Culture-treated Cultureware. An equal number of cells were plated on Corning BioCoat Poly-D-Lysine 384-well black/clear (right) and 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 Corning BioCoat Poly-D-Lysine coated wells and the Falcon Tissue Culture-treated wells. After washing, using a Skatron Washer (Molecular Devices) (middle), the cells remained attached to the Corning BioCoat Poly-D-Lysine wells while few cells remained attached to the 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 Cul	ture Reagents	

Extracellular Matrix Proteins

356236	Collagen I, rat tail	10 x 100 mg		
356234	Corning Matrigel [®] M	latrix 5 mL		
Cell Recovery Reagents				
354235	Dispase	100 mL		
354253	Cell Recovery Solutio	n 100 mL		

Cell Culture Tools

Corning BioCoat Collagen I Cellware				
354485	75 cm ² vented-cap Flasks	5		
Corning	BioCoat Poly-D-Lysine Cel	ware		
354469	100 mm Dishes	10		
Intestina	Cell Environments Intestinal Epithelium Differentiation Environment			
355057	Intestinal Epithelium Differentiation Environment	1 t		
Corning	BioCoat HTS Caco-2 Assay	Systems		
354801	Corning BioCoat Fibrillar Co 24-Multiwell Insert System media to perform 24 individ three-day Caco-2 assays	plus		
355357	Differentiation 2 > Medium	250 mL		
355058	Intestinal Epithelium Differentiation Media Pack	1 kit		
355006	MITO+ Serum Extender	5 mL		
000000	MITOT Serun Extender	5 IIIL		

Membrane Insert Systems

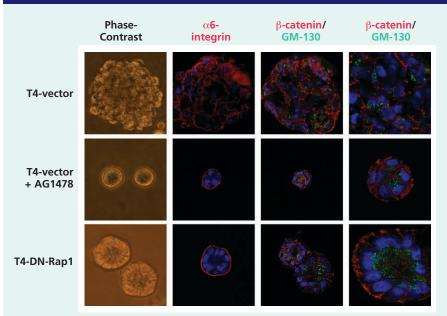
Corning BioCoat Fibrillar Collagen Cell Culture Inserts			
354472	1.0 µm inserts in four 6-well plates	24	

Collagen 24-Multiwell Insert

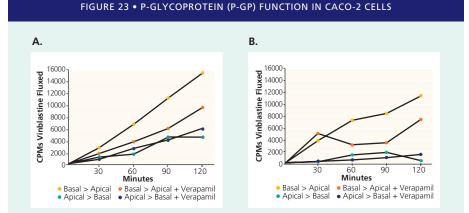
System plus media to perform 24 individual three-day Caco-2 assays

For a complete product listing, see page 19.

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

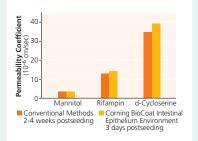


Corning Matrigel Matrix Growth Factor Reduced supports mammary acini formation *in vitro*. Malignant T4-2 cells were grown in three-dimensional culture on Corning Matrigel Matrix Growth Factor Reduced. Cells were stably transfected with control (T4-vector) or dominant negative-Rap1 (T4-DN-Rap1). Inhibition 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 (α 6-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):4759-4766⁵¹. Reproduced with permission.



Caco-2 cells were cultured using the three-day Corning 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 ³H-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 μ M verapamil was added to the insert chambers.

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

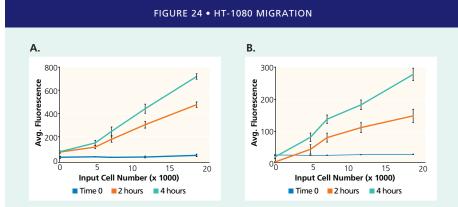


Barrier formation occurs three days postseeding in the Corning BioCoat™ Intestinal Epithelium Differentiation Environment and two to four weeks with conventional methods. Monolayers formed using either the Corning BioCoat Intestinal Epithelium Differentiation Environment or conventional methods are equally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

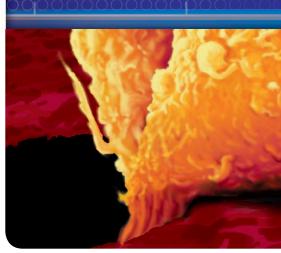
Otv

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 Falcon® Cell Culture Inserts or Corning® 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 Corning 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.



Migration of Calcein AM (A) and DilC₁₂(3) (B) labeled human fibrosarcoma cells (HT-1080) through Corning 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 DilC₁₂(3). Values represent the mean of 8 wells \pm S.D. Migration from as few as 4,000 input cells can be detected.



Pseudo-colored image for illustrative purposes only.

Tools for Tumor Cell Culture

Description

at No

Cat. No.	Description	Qty.		
Cell Culture Reagents				
Extracell	ular Matrix Proteins			
354248	Corning Matrigel Matrix, High Concentration	10 mL		
Cell Reco	overy Reagents			
354253	Cell Recovery Solution	100 mL		
354235	Dispase	100 mL		
Fluorescent Dyes				
354216	Calcein AM	10 x 50 µg		
354218	DilC ₁₂ (3)	100 mg		
Membrane Incert Systems				

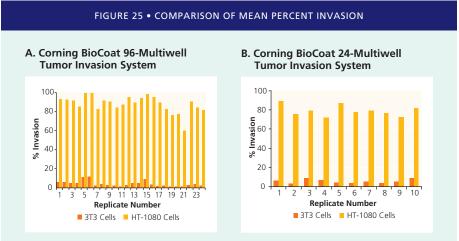
Membrane Insert Systems

Corning BioCoat Matrigel Invasion Chambers			
354480	8.0 µm inserts in two 24-well plates	24	
Corning	BioCoat Tumor Invasion	System	
354165	One insert plate with 24-well plate and lid	1	
Falcon Cell Culture Inserts			
351182	3.0 µm pore size with 24-well plate and lid	1	

For a complete product listing, see page 19.

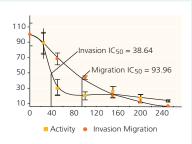
👌 DID YOU KNOW?

 Corning offers a full range of dishes and flasks. Please contact your sales representative for more information. The Corning[®] BioCoat[™] Matrigel[®] Invasion Chambers and Corning BioCoat Tumor Invasion Systems are optimized systems that utilize standardized coating procedures to ensure even coating of Corning Matrigel Matrix for reproducible results (**Figure 25**). The Corning 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, Corning Matrigel Matrix can be used to help support tumor cell engraftment in mice⁶⁴⁻⁶⁶. These tools allow researchers to dissect various areas of tumor biology, from analysis of signaling pathways *in vitro* to *in vivo* tumor formation.



Multiple lots of the Corning BioCoat 96-Multiwell Tumor Invasion System and Corning BioCoat 24-Multiwell Tumor Invasion System were assayed to show reproducibility with these systems. Multiple lots of Corning BioCoat 96-Multiwell Tumor Invasion System (A) and Corning 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 (Corning BioCoat 96-Multiwell Tumor Invasion System) or a CytoFluor[®] plate reader (Corning BioCoat 24-Multiwell Tumor Invasion System). Mean percent invasion of NIH-3T3 and HT-1080 cells were compared. Cells were labeled post-invasion using Corning Calcein AM.

FIGURE 26 • INHIBITION OF PC3 MIGRATION AND INVASION BY DOXYCYCLINE



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

Cell Culture Reagents

Extracellular Matrix Proteins

	DESCRIPTION	QTY./CASE	CAT. NO.
Corning [®] Matrigel [®]	Corning Matrigel Matrix	5 mL	356234
Basement Membrane Matrix	Corning Matrigel Matrix	10 mL	354234
	Corning Matrigel Matrix (50 mL)	5 x 10 mL	356235
	Corning Matrigel Matrix High Concentration (HC)	10 mL	354248
	Corning Matrigel Matrix Phenol Red-Free	10 mL	356237
	Corning Matrigel Matrix HC Phenol Red-free	10 mL	354262
	Corning Matrigel Matrix Growth Factor Reduced (GFR)	5 mL	356230
	Corning Matrigel Matrix GFR	10 mL	354230
	Corning Matrigel Matrix HC GFR	10 mL	354263
	Corning Matrigel hESC- qualified Matrix	5 mL	354277
	Corning Matrigel Matrix Phenol Red-free GFR	10 mL	356231
Fibronectin	Fibronectin, human	1 mg	354008
	Fibronectin, human	5 mg	356008
	Fibronectin, human (25 mg)	5 x 5 mg	356009
Collagen I	Collagen I, bovine	30 mg	354231
	Collagen I, human	0.25 mg	354243
	Collagen I, human	10 mg	354265
	Collagen I, rat tail	100 mg	354236
	Collagen I, rat tail (1 g)	10 x 100 mg	356236
	Collagen I, human recombinant	250 ug	354254
Laminin	Laminin, mouse	1 mg	354232
	Ultra-pure Laminin, mouse	1 mg	354239
	Laminin/Entactin Complex High Concentration	10.5 mg	354259
Poly-D-Lysine	Poly-D-Lysine, synthetic	20 mg	354210
Corning PuraMatrix [™]	Peptide Hydrogel, synthetic	5 mL	354250

Cytokines and Media Additives

	DESCRIPTION	QTY./CASE	CAT. NO.
Epidermal Growth	Mouse, natural (culture grade)	100 µg	354001
Factor (EGF)	Mouse, natural (culture grade) (10 x 100 μg)	1 mg	356001
	Mouse, natural (receptor grade)	100 µg	354010
	Mouse, natural (receptor grade) (5 x 100 µg)	500 µg	356010
	Human recombinant	100 µg	354052
	Human recombinant (10 x100 µg)	1 mg	356052
Basic Fibroblast	bFGF, bovine natural	10 µg	356037
Growth Factor (bFGF)	bFGF, human recombinant	10 µg	354060
	bFGF, human recombinant (50 µg)	5 x 10 µg	356060
	bFGF, human recombinant (100 μg)	10 x 10 µg	356061
ITS Universal Culture	5 liter equivalent	5 mL	354351
Supplement Premix	20 liter equivalent	20 mL	354350
Nerve Growth Factor	2.5S NGF, mouse natural	10 µg	354005
(NGF)	2.5S NGF, mouse natural	100 µg	356004
	2.5S NGF, mouse natural (1 mg)	2 x 500 µg	356005
	7S NGF, mouse natural	100 µg	354009
Vascular Endothelial Growth Factor (VEGF)	Human recombinant	10 µg	354107
MITO+ Serum Extender	5 liter equivalent	5 mL	355006
Endothelial Cell	Bovine	15 mg	354006
Growth Supplement (ECGS)	Bovine	100 mg	356006
Specialty Media	E-STIM Endothelial Cell Culture Medium	500 mL	355054
	Hepatocyte Culture Media	500 mL	355056
	Intestinal Differentiation Media Pack	1 pack	355058
	Enterocyte Differentiation Medium	2 x 250 mL	355357
HUVEC-2 Cells	HUVEC-2 Cells	1 cryovial	354151

Corning Cell Recovery/Detachment Reagents

Cell Recovery	Dispase	100 mL	354235	
Reagents	Cell Recovery Solution	100 mL	354253	
Corning Fluorescent Dyes				
Fluorescent Dyes	Calcein AM Fluorescent Dye	10 x 50 µg	354216	
	Calcein AM Fluorescent Dye	1 mg	354217	
	DiIC ₁₂ (3) Fluorescent Dye	100 mg	354218	

Cell Culture Tools

Corning[®] BioCoat[™] Collagen I Cellware

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150 mm culture dishes 5 354551 25 cm² vented-cap flasks 10 354484 25 cm² vented-cap flasks (5 sleeves of 10) 50 356484 75 cm² vented-cap flasks (10 sleeves of 5) 50 356485 75 cm² vented-cap flasks (10 sleeves of 5) 50 356485 150 cm² vented-cap flasks 50 356485 150 cm² vented-cap flasks 51 354486 150 cm² vented-cap flasks (8 sleeves of 5) 40 356486 Coverslips 22 mm round No.1 German glass 60 354089	100 mm culture dishes	10	354450
25 cm² vented-cap flasks 10 354484 25 cm² vented-cap flasks (5 sleeves of 10) 50 356484 75 cm² vented-cap flasks 5 354485 75 cm² vented-cap flasks (10 sleeves of 5) 50 356485 150 cm² vented-cap flasks 5 354486 150 cm² vented-cap flasks 8 sleeves of 5) 40 356486 Coverslips 22 mm round No.1 German glass 60 354089	100 mm culture dishes (4 sleeves of 10)	40	356450
25 cm² vented-cap flasks (5 sleeves of 10) 50 356484 75 cm² vented-cap flasks 5 354485 75 cm² vented-cap flasks (10 sleeves of 5) 50 356485 150 cm² vented-cap flasks 5 354486 150 cm² vented-cap flasks (8 sleeves of 5) 40 356486 Coverslips 22 mm round No.1 German glass 60 354089	150 mm culture dishes	5	354551
75 cm² vented-cap flasks 5 354485 75 cm² vented-cap flasks (10 sleeves of 5) 50 356485 150 cm² vented-cap flasks 5 354486 150 cm² vented-cap flasks (8 sleeves of 5) 40 356486 Coverslips 22 mm round No.1 German glass 60 354089	25 cm ² vented-cap flasks	10	354484
75 cm² vented-cap flasks (10 sleeves of 5) 50 356485 150 cm² vented-cap flasks 5 354486 150 cm² vented-cap flasks (8 sleeves of 5) 40 356486 Coverslips 22 mm round No.1 German glass 60 354089	25 cm ² vented-cap flasks (5 sleeves of 10)	50	356484
150 cm² vented-cap flasks 5 354486 150 cm² vented-cap flasks (8 sleeves of 5) 40 356486 Coverslips 22 mm round No.1 German glass 60 354089	75 cm ² vented-cap flasks	5	354485
150 cm² vented-cap flasks (8 sleeves of 5)40356486Coverslips 22 mm round No.1 German glass60354089	75 cm ² vented-cap flasks (10 sleeves of 5)	50	356485
Coverslips 22 mm round No.1 German glass 60 354089	150 cm ² vented-cap flasks	5	354486
	150 cm ² vented-cap flasks (8 sleeves of 5)	40	356486
	Coverslips 22 mm round No.1 German glass	60	354089
4-well CultureSlides 12 354557	4-well CultureSlides	12	354557
8-well CultureSlides 12 354630	8-well CultureSlides	12	354630

Corning BioCoat Poly-D-Lysine Cellware

DESCRIPTION	QTY./CASE	CAT. NO
6-well plates	5	354413
6-well plates (10 sleeves of 5)	50	356413
12-well plates	5	354470
12-well plates (10 sleeves of 5)	50	356470
24-well plates	5	354414
24-well plates (10 sleeves of 5)	50	356414
48-well plates	5	354509
48-well plates (10 sleeves of 5)	50	356509
96-well plates	5	354461
96-well plates (10 sleeves of 5)	50	356461
96-well plates	80	356690
96-well black/clear plates	5	354640
96-well black/clear plates (10 sleeves of 5)	50	356640
96-well black/clear plates	80	356692
96-well white/clear plates	5	354651
96-well white/clear plates (10 sleeves of 5)	50	356651
96-well white/clear plates	80	356693
96-well white plates	5	354620
96-well white plates (10 sleeves of 5)	50	356620
96-well white/opaque plates	80	356691
35 mm culture dishes	20	354467
35 mm culture dishes (5 sleeves of 20)	100	356467
60 mm culture dishes	20	354468
60 mm culture dishes (5 sleeves of 20)	100	356468
100 mm culture dishes	10	354469
100 mm culture dishes (4 sleeves of 10)	40	356469
150 mm culture dishes	5	354550
25 cm ² vented-cap flasks	10	354536
25 cm ² vented-cap flasks (5 sleeves of 10)	50	356536
75 cm ² vented-cap flasks	5	354537
75 cm ² vented-cap flasks (10 sleeves of 5)	50	356537
150 cm ² vented-cap flasks	5	354538
150 cm ² vented-cap flasks (8 sleeves of 5)	40	356538
Coverslips 12 mm round No.1 German glass	80	354086
35 mm Coverslip-bottom dishes No. 1 German glass	20	354077
4-well CultureSlides	12	354577
8-well CultureSlides	12	354632

Corning[®] BioCoat[™] Poly-L-Lysine Cellware

DESCRIPTION	QTY./CASE	CAT. NO
6-well plates	5	354515
6-well plates (10 sleeves of 5)	50	356515
96-well plates	5	354516
96-well plates (10 sleeves of 5)	50	356516
35 mm culture dishes	20	354518
35 mm culture dishes (5 sleeves of 20)	100	356518
60 mm culture dishes	20	354517
60 mm culture dishes (5 sleeves of 20)	100	356517
Coverslips 12 mm round No.1 German glass	80	354085

Corning BioCoat Laminin Cellware

DESCRIPTION	QTY./CASE	CAT. NO
6-well plates	5	354404
12-well plates	5	354502
24-well plates	5	354412
48-well plates	5	354507
96-well plates	5	354410
35 mm culture dishes	20	354458
60 mm culture dishes	20	354405
100 mm culture dishes	10	354452
150 mm culture dishes	5	354553
25 cm ² plug-seal flasks	10	354533
75 cm ² plug-seal flasks	10	354522

Corning BioCoat Matrigel[®] Matrix – for Hepatocytes

DESCRIPTION	QTY./CASE	CAT. NO
6-well plates	5	354510

Corning BioCoat Matrigel Matrix Plates for Embryonic Stem Cell Culture

DESCRIPTION	QTY./CASE	CAT. NO
6-well plates	5	354671

Corning BioCoat Poly-D-Lysine/Laminin Cellware

DESCRIPTION	QTY./CASE	CAT. NO
6-well plates	5	354595
24-well plates	5	354619
96-well plates	5	354596
100 mm culture dishes	10	354455
Coverslips 12 mm round No.1 German glass	80	354087
2-well CultureSlides	12	354687
8-well CultureSlides	12	354688

Corning BioCoat Poly-L-Ornithine/Laminin Cellware

	CAT. NO
5	354658
5	354659
5	354657
	5 5 5

Falcon[®] Cultureware

	DESCRIPTION	QTY./CASE	CAT. NO.
4-well CultureSlides	1.7 cm ² growth surface area per well	96	354104
		24	354114
8-well CultureSlides	0.7 cm ² growth surface area per well	96	354108
		24	354118
96-well Plate	Black/Clear, with lid	32	353219

Primaria[™] Cultureware

	DESCRIPTION	QTY./CASE	CAT. NO.
Corning Primaria [™] Cell Culture Dishes with lid	35x10 mm style Easy-Grip	200	353801
	60x15 mm style	200	353802
	100x20 mm style	200	353803
Corning Primaria Cell Culture Flasks	25 cm ² growth area, 50 mL, canted neck	200	353813
with plug-seal screw cap	75 cm ² growth area, 250 mL straight neck	100	353824
Corning Primaria Cell Culture Flasks with 0.2 µm membrane vented screw cap	25 cm ² growth area, 50 mL, canted neck	100	353808
	75 cm ² growth area, 250 mL, straight neck	100	353810
Corning Primaria Cell Culture Plates, flat-bottom with lid	6-well	50	353846
	24-well	50	353847
	96-well	50	353872

Corning[®] Gentest[™] Hepatocytes and Reagents

	DESCRIPTION	QTY./CASE	CAT. NO.
Cholyl-lysyl-Fluorescein (CLF)	Hepatocyte Bile Acid Transporter Uptake	1 mg	451041
Cryopreserved Hepatocyte Purification Kit	Allows purification of six individual 1.5 mL cryotubes	1 kit	454500
Hepatocyte One-Step Purification Kit	Allows purification of four individual 1.5 mL cryotubes	1 kit	454600
High Viability Recovery Kit		1 kit	454534
High Viability Recovery Medium	5 mg/mL protein	45 mL	454560
Plating Medium	5 mg/mL protein	45 mL	454561
Culture Media Kit		500 mL	455056
Fresh Human Hepatocytes	One Million Human Hepatocytes in Suspension	1 million cells/vial (10 million cells minimum order)	454401
	6-well plate	12 million cells per Collagen I plate	454406
	12-well plate	9.6 million cells per Collagen I plate	454412
	24-well plate	9.6 million cells per Collagen I plate	454424
	48-well plate	7.2 million cells per Collagen I plate	454425
	96-well plate	4.8 million cells per Collagen I plate	454496
	6-well plates with Matrigel Overlay	12 million cells per Collagen I plate	454480
	12-well plates with Matrigel Overlay	9.6 million cells per Collagen I plate	454481
	24-well plates with Matrigel Overlay	9.6 million cells per Collagen I plate	454482
	48-well plates with Matrigel Overlay	7.2 million cells per Collagen I plate	454483
	96-well plates with Matrigel Overlay	4.8 million cells per Collagen I plate	454484
	25 cm ² flask	5 million cells per Collagen I flask	454415
	75 cm² flask	15 million cells per Collagen I flask	454475

Transporter-Qualified Human CryoHepatocytes

DESCRIPTION	QTY./CASE	CAT. NO.
\leq 5 million cells/vial	1.5 mL	454541
2 millions cells/vial	1.5 mL	454426
>5 million cells/vial	1.5 mL	454427
100 tests	1000 assay points	454460
2 million cells/vial	1.5 mL	454550
>5 million cells/vial	1.5 mL	454551
	 ≤5 million cells/vial 2 millions cells/vial >5 million cells/vial 100 tests 2 million cells/vial 	 ≤5 million cells/vial 2 millions cells/vial 55 million cells/vial 1.5 mL 100 tests 1000 assay points 2 million cells/vial 1.5 mL

Metabolism-Qualified Human CryoHepatocytes

	DESCRIPTION	QTY./CASE	CAT. NO.
Human Plateable Metabolisim-Qualified	≥5 million cells/vial	1.5 mL	454543
Human Metabolism-Qualified	2-5 million cells	1.5 mL	454503
Human Metabolism-Qualified in Suspension	>5 million cells/vial	1.5 mL	454504

Cell Environments

	DESCRIPTION	QTY./CASE	CAT. NO
Corning BioCoat™ Tumor Invasion System	One insert plate with one 24-well plate and lid	1	354165
	Five insert plates with five 24-well plates and lids	5	354166
	One insert plate with one 96-well plate and lid	1	354167
	Five insert plates with five 96-well plates and lids	5	354168
Corning BioCoat Angiogenesis System: Endothelial Cell Invasion	One insert plate with one 24-well plate and lid	1	354141
	Five insert plates with five 24-well plates and lids	5	354142
Corning BioCoat Angiogenesis System: Endothelial Cell Migration	One insert plate with one 24-well plate and lid	1	354143
	Five insert plates with five 24-well plates and lids	5	354144
	One insert plate with one 96-well plate and lid	1	354147
	Five insert plates with five 96-well plates and lids	5	354148
Corning BioCoat Angiogenesis System:	96-well Black/Clear Microplate	1	354149
Endothelial Tube Formation	96-well Black/Clear Microplate	5	354150
Corning BioCoat Matrigel [®] Invasion Chambers	8.0 µm inserts in four 6-well plates	24	354481
	8.0 µm inserts in two 24-well plates	24	354480
Corning BioCoat GFR Matrigel Invasion Chambers	8.0 µm inserts in two 24-well plates	24	354483
Corning BioCoat Endothelial Cells	Endothelial Cell Growth Environment	1	355053
Corning BioCoat Hepatocyte Differentiation	Hepatocyte Differen- tiation Environment	1	355055
Corning BioCoat Intestinal Epithelial Differentiation Environment	Intestinal Epithelium Differentiation Envi- ronment	1	355057

Cell Environments (continued)

	DESCRIPTION	QTY./CASE	CAT. NO.
Corning BioCoat HTS Caco-2 Assay Systems	1.0 μm inserts in one 24-Multiwell plate with feeder tray and lid	1	354801
	1.0 μm inserts in one 24-Multiwell plate with feeder tray and lid	5	354802
Corning BioCoat Fibrillar Collagen 24-Multiwell Insert Systems	1.0 μm inserts in one 24-Multiwell plate with feeder tray and lid	1	354803
	1.0 μm inserts in one 24-Multiwell plate with feeder tray and lid	5	354804

Membrane Insert Systems

For use with Falcon[®] Cell Culture Insert Companion Plates

	DESCRIPTION	QTY./CASE	CAT. NO.
0.4 μm, Transparent PET membrane	for 6-well plates	48	353090
	for 12-well plates	48	353180
	for 24-well plates	48	353095
1.0 µm, Transparent PET membrane	for 6-well plates	48	353102
membrane	for 12-well plates	48	353103
	for 24-well plates	48	353104
3.0 µm, Transparent PET membrane	for 6-well plates	48	353091
memprane	for 12-well plates	48	353181
	for 24-well plates	48	353096
0.4 µm, HD inserts	for 6-well plates	48	353493
Translucent PET membrane	for 12-well plates	48	353494
	for 24-well plates	48	353495
3.0 µm HD Inserts,	for 6-well plates	48	353092
Translucent PET membrane	for 12-well plates	48	353292
	for 24-well plates	48	353492
8.0 µm Translucent PET	for 6-well plates	48	353093
membrane	for 12-well plates	48	353182
	for 24-well plates	48	353097
Falcon Cell Culture	6-well plate	50	353502
Insert Companion Plates	12-well plate	50	353503
	24-well plate	50	353504
Falcon 24-Multiwell	1.0 µm PET membrane	1	351180
Insert Systems	1.0 µm PET membrane	5	351181
	3.0 µm PET membrane	1	351182
	3.0 µm PET membrane	5	351183
	8.0 µm PET membrane	1	351184
	8.0 µm PET membrane	5	351185
Falcon 24-Multiwell Insert Systems	Feeder tray with lid	5	351186
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Membrane Insert Systems (continued)

Nembrane Insert Systems (continued)			
	DESCRIPTION	QTY./CASE	CAT. NO.
Falcon 96-Multiwell Insert Systems	One insert plate with feeder tray and lid	1	351130
	Five insert plates with feeder trays and lids	5	351131
	Five insert plates with 96-square well, angled- bottom plates and lids	5	353938
Falcon 96-well Square Well, Angled-Bottom Plate and Lid	96-square well, angled bottom plate and lid	5	353925
Falcon 96-well Feeder Tray and Lid	Falcon feeder trays and lids	5	353924
Corning FluoroBlok™ 96-Multiwell Insert	3.0 µm, One insert plate with 96-well plate and lid	1	351161
Systems	3.0 $\mu m,$ Five insert plates with 96-well plates and lids	5	351162
	8.0 µm, One insert plate with 96-well plate and lid	1	351163
	$8.0\ \mu\text{m},$ Five insert plates with 96-well plates and lids	5	351164
Falcon 96-Square Well, Flat-Bottom Plate and Lid	96-square well, flat- bottom plate and lid	5	353928
Corning BioCoat™ Collagen I	0.4 µm inserts in four 6-well plates	24	354442
Cell Culture Inserts	0.4 µm inserts in two 24-well plates	24	354444
	1.0 μm inserts in four 6-well plates	24	354580
	1.0 μm inserts in two 24-well plates	24	354482
	3.0 μm inserts in four 6-well plates	24	354540
	3.0 µm inserts in two 24-well plates	24	354541
Corning BioCoat Collagen IV	1.0 μm inserts in two 24-well plates	24	354591
Cell Culture Inserts	3.0 μm inserts in four 6-well plates	24	354544
	3.0 μm inserts in two 24-well plates	24	354545
Corning BioCoat Fibrillar Collagen Cell Culture	1.0 μm inserts in four 6-well plates	24	354472
Inserts	1.0 μm inserts in two 24-well plates	24	354474
Corning BioCoat Fibronectin	0.4 µm inserts in four 6-well plates	24	354440
Cell Culture Inserts	0.4 µm inserts in two 24-well plates	24	354445
	3.0 µm inserts in two 24-well plates	24	354543

Membrane Insert Systems (continued)

	DESCRIPTION	QTY./CASE	CAT. NO
	DESCRIPTION		
Corning BioCoat FluoroBlok Fibronectin Cell Culture Inserts	3.0 µm inserts in two 24-well plates	24	354597
Corning BioCoat Collagen I 24-Multiwell Insert System	3.0 µm insert plate with 24-well plate and lid	1	354598
Corning [®] BioCoat™ Control Cell Culture Inserts	0.4 µm inserts in four 6-well plates	24	354570
	0.4 µm inserts in two 24-well plates	24	354572
	1.0 µm inserts in four 6-well plates	24	354567
	1.0 µm inserts in two 24-well plates	24	354569
	3.0 µm inserts in four 6-well plates	24	354573
	3.0 µm inserts in two 24-well plates	24	354575
	8.0 µm inserts in four 6-well plates	24	354576
	8.0 µm inserts in two 24-well plates	24	354578
Corning FluoroBlok [™]	1.0 µm inserts	48	351150
Cell Culture Inserts For use with Falcon [®] 24-well Cell	3.0 µm inserts	48	351151
Culture Insert Companion Plates (Cat. No. 353504)	8.0 µm inserts	48	351152
Corning FluoroBlok 24-Multiwell Insert Systems	1.0 µm insert system in one 24- well plate	1	351153
	1.0 µm insert system in one 24- well plate	5	351154
	3.0 µm insert system in one 24- well plate	1	351155
	3.0 µm insert system in one 24- well plate	5	351156
	8.0 µm insert system in one 24- well plate	1	351157
	8.0 µm insert system in one 24- well plate	5	351158
Corning BioCoat Deep-Well Plates For use with Corning BioCoat Cell Culture Inserts	6-well Deep-Well Plates	4	355467

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