

Corning® BioCoat™ Cultureware

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About the cover:

Computer colorization of, from top to bottom:

- ▶ Rat cerebellar granule (RCG) cells cultured on Corning BioCoat Poly-D-Lysine (PDL) cultureware (page 16)
- ▶ Human umbilical vein endothelial cells (HUVECs) grown for 7 days on Corning BioCoat Gelatin 6-well multiwell plates (page 7)
- ▶ BHK-21 fibroblasts cultured on Corning BioCoat Fibronectin cultureslides (page 10)
- ▶ Neurotransmitter induced calcium waves in astrocytes plated on Corning BioCoat Laminin/Fibronectin cultureware (page 13)

Introduction

The development and normal functioning of cells depends on interactions with molecules in their micro-environment. 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 is composed of a number of different macromolecules whose structural integrity and functional composition are important in maintaining normal tissue architecture, in development and in tissue-specific function. The ECM exerts influences on behavior (adherence, spreading, differentiation, and migration) and the pattern of gene expression of the cells in contact with it. The ECM, however, is not static but changes during both normal development and in tissue repair and regeneration and is intimately involved in both normal biological function and response to injury¹.

To create physiologically relevant *in vitro* models that support normal cell culture and function, the components of the *in vivo* environment must be incorporated. The use of an ECM as a coating for tissue culture surfaces permits the development of model systems which closely mimic *in vivo* conditions. The choice of ECM is an important component to consider when optimizing *in vitro* culture systems.

Corning® BioCoat™ Cultureware

Corning BioCoat cultureware is a unique line of tissue culture vessels with various ECM components applied to vessel surfaces by a proprietary manufacturing process. The result is a uniform, optically clear matrix substrate. This technology, together with our exacting quality control, guarantees the performance of each lot, as well as consistency from lot-to-lot.

Corning BioCoat cultureware promotes cell attachment, spreading, growth, and differentiation of a variety of primary cells and cell lines in serum-free or serum-containing cultures.

Applications include:

- ▶ Cell adhesion and co-culture assays
- ▶ Receptor-ligand-binding assays
- ▶ Routine drug screening assays
- ▶ Studies of tissue morphogenesis
- ▶ Studies of cell-matrix interactions
- ▶ Regulation of signal transduction and gene expression
- ▶ Cell migration and invasion assays
- ▶ Angiogenesis studies
- ▶ Studies of transport and permeability



Get the Corning BioCoat Advantages

Ready-to-Use Convenience

Spend more time performing your experiments rather than preparing for them. Precoated BioCoat cultureware saves time and labor costs while increasing productivity.

Quality Assurance Testing

BioCoat cultureware is thoroughly tested for bioactivity and guaranteed to perform as claimed so you can use with complete confidence.

Reliable Performance

BioCoat cultureware improves cell attachment and increases proliferation rates for a variety of normal and transformed cells.

Lot-to-Lot Consistency

Corning prides itself on maintaining highly controlled ISO 9001 and cGMP production environments and validated manufacturing procedures that result in uniformity and consistent performance.

Wide Selection

Available with a wide range of ECM proteins and attachment factors, BioCoat cultureware helps optimize conditions for attachment, growth, or differentiation for your cell type.

Readily Available

BioCoat cultureware is available from stock for immediate shipments.

1. Alberts, B., et al., Mol. Bio. of the Cell (Third Edition). Garland Publishing, NY (1994).

Corning® BioCoat™ Cultureware Vessel Options

Corning is a world leader in providing researchers with top-quality cell culture products. Corning BioCoat cultureware is manufactured to ensure consistent, reliable results. Trust Corning, the first name in cell culture.



Flasks are available in various sizes and designs to meet all of your cell culture needs in standard, plug-seal, and vented cap options.



Multiwell Plates are manufactured using a crystal-grade polystyrene and feature a patented labyrinth lid, condensation rings, and deep well design to control contamination while reducing evaporation and minimizing edge effects.



Dishes are exceptionally flat for distortion-free optics and feature stacking rings for easier stacking and handling.



CultureSlides have an innovative sealing design that minimizes leakage and a plastic chamber affixed to a specially cleaned glass slide that can be removed with an easy-to-use disposable safety removal tool.



Coverslips are No. 1 German glass and provide an optically clear surface which is non-neurotoxic and exhibits low background fluorescence. The convenient package also acts as a storage container and allows for easy coverslip manipulation.



Coverslip-bottom Dishes are 35 mm dishes with a coverslip-bottom that is easy to use and facilitates preparation of cells for microscopic analysis. The coverslip floor is No. 1 German glass. This format is ideal for use in high resolution and inverted microscopy, fluorescence imaging in live cells, confocal microscopy, phase contrast microscopy, and micro-manipulations.



Cell Culture Inserts contain a microporous membrane that mimics the *in vivo* cell environment by allowing cells to be fed both apically and basolaterally. BioCoat inserts come in a variety of formats, membrane types, and surfaces.



Microplates have been further enhanced (versus TC) with biological coatings of highly purified extracellular matrix (ECM) proteins. It features superior lot-to-lot and intra-well consistency, minimized cross-talk, versatility of plate colors, and a stackable design.

Corning® BioCoat™ Manufacturing Facilities



Corning has a highly controlled manufacturing environment for Corning BioCoat products in Bedford, MA and its ISO 9001, current Good Manufacturing Practices (cGMP) certified plant in Kennebunk, ME. ISO certification verifies that the facility meets international quality standards and that Corning provides assurance to customers that it is totally committed to delivering superior quality and product improvements.

All Corning BioCoat products are produced under aseptic conditions to minimize the risk of product contamination from bacteria, fungi, and particulates.

Proprietary manufacturing technology, validated procedures, and strict compliance with established protocols, combined with Corning exacting quality control, assure the biological performance of each lot, as well as consistency from lot-to-lot.

Proprietary formulation and manufacturing techniques allow Corning to produce room temperature Collagen I, Gelatin, and Poly-Lysine cultureware. Extensive accelerated and real-time studies in the laboratory have confirmed product performance for at least one year under dry conditions at temperatures from 4°C to 30°C.

Corning® BioCoat™ Collagen I Cultureware

Collagen I, found in most tissues and organs, is most plentiful in dermis, tendon, and bone. It is an integral part of the framework that holds cells and tissues together and has been recognized as a useful matrix for improving cell culture. *In vitro* use of collagen can exert effects on the adherence, morphology, growth, migration, and differentiation of a variety of cell types¹.

Applications include:

- ▶ Promotion of cell attachment and spreading
- ▶ Rapid expansion of cell populations
- ▶ Serum-free or reduced serum culture
- ▶ Cell adhesion assays
- ▶ Improving survival of primary cells in culture

Has been used to culture:

- ▶ Primary murine cardiac myocytes²
- ▶ Human vascular SMC³
- ▶ PC12 cells and SH-SY5Y cells⁴
- ▶ Mouse primary keratinocytes⁵
- ▶ SK-MEL-28-N1 cells⁶
- ▶ Murine myoblast C2C12 cells⁷
- ▶ HUVEC⁸
- ▶ HEK-293 cells⁹
- ▶ Rat Kupffer cells¹⁰
- ▶ MDA-231 breast cancer cells¹¹

Source

Rat tail tendon

Quality Control

- ▶ Tested for ability to promote attachment and spreading of HT-1080 human fibrosarcoma cells
- ▶ Tested and found negative for bacteria and fungi
- ▶ Collagen I purity >90% by SDS-PAGE

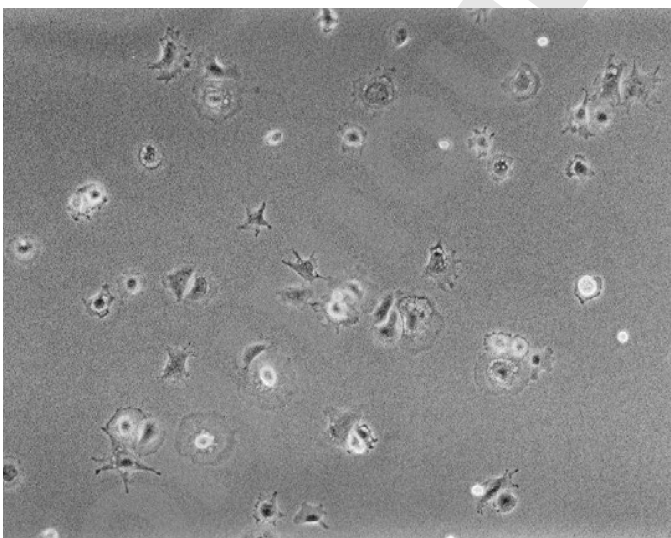
Storage

4°C to 30°C under dry conditions.

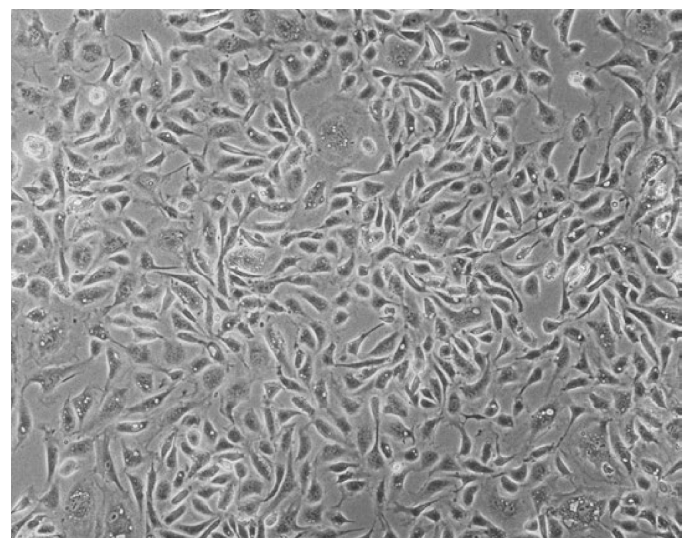
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9. Smith, J.S., et al., *J. Neurosci.* 21(4):1096 (2001).
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Effects of Corning BioCoat™ Collagen I Cultureware on Fetal Bovine Heart Endothelial (FBHE) Cells



FBHE cells grown for five days in basal medium containing 10% FBS on tissue culture plastic show sparse growth.



FBHE cells grown for five days using the Corning BioCoat Endothelial cell growth environment (Collagen I cultureware) form a confluent monolayer and show numerous mitotic cells.

Corning® BioCoat™ Gelatin Cultureware

Corning BioCoat Gelatin cultureware provides an attachment and growth promoting substrate for the culture of a variety of cell types. Gelatin is commonly used in the culture of vascular endothelial cells, muscle, embryonic stem (ES) cells, and F9 teratocarcinoma cells. It is also suitable for promoting adhesion of transfected cell types. Gelatin is a heterogeneous mixture of water-soluble proteins derived through the hydrolysis of Collagen.

Applications include:

- ▶ Promotion of cell attachment and spreading
- ▶ Culture of normal and transfected F9 teratocarcinoma cells for gene expression studies⁶
- ▶ Culture of HUVEC for E-Selectin⁷ expression and VEGF induction⁸

Has been used to culture:

- ▶ HUVEC⁷, BME¹, BAEC²
- ▶ ES cells³
- ▶ C2C12⁴ and MM14⁵ myoblasts
- ▶ Normal and transfected F9 teratocarcinoma cells

Source

Gelatin, porcine

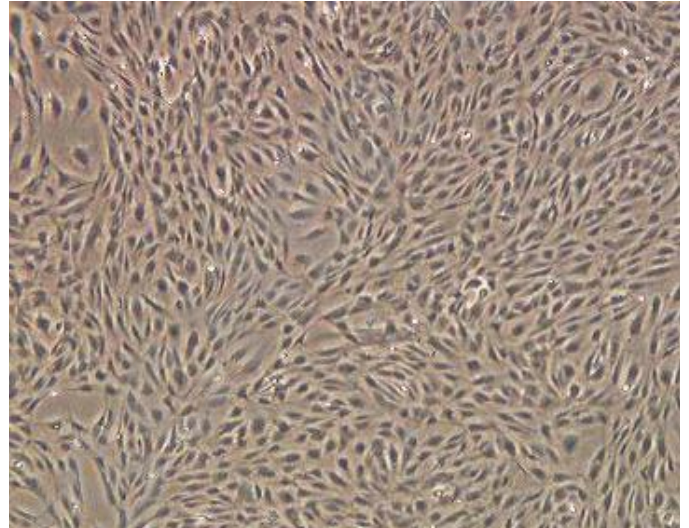
Quality Control

- ▶ Tested for ability to promote proliferation of HUVECs
- ▶ Tested and found negative for bacteria and fungi

Storage

4°C to 30°C under dry conditions.

Effect of Corning BioCoat Gelatin Cultureware on Human Umbilical Vein Endothelial Cells (HUVEC)



HUVEC grown for seven days on Corning BioCoat Gelatin 6-well multiwell plates seeded at a density of 2×10^4 in the presence of Corning Endothelial cell culture medium.

References

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6. Laurance, M.E., et al., J. Biol. Chem. 272(5):2646 (1997).
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Corning® BioCoat™ Poly-Lysine Cultureware

Poly-D-Lysine (PDL) and Poly-L-Lysine (PLL) are synthetic compounds that enhance cell adhesion and protein absorption by altering surface charges on the culture substrate. In addition to promoting cell adhesion, poly-lysine surface treatments support neurite outgrowth and improve the survival of many central nervous system (CNS) primary cells in culture. As PDL and PLL are synthetic molecules, they do not stimulate biological activity in the cells cultured on them, and they do not introduce impurities carried by natural polymers.

Applications include:

- ▶ Attachment and spreading of a variety of cell types
- ▶ Cell differentiation and neurite outgrowth
- ▶ Attachment of fastidious transfected cell lines
- ▶ Support survival of primary neurons in culture
- ▶ Serum-free or reduced-serum culture

Has been used to culture:

- ▶ Primary mouse brain capillaries¹
- ▶ HEK-293 cells²⁻⁵
- ▶ MDA-231 breast cancer cells⁶
- ▶ Mouse cerebellar granule neurons⁷
- ▶ Transfected rat 1 cells⁸
- ▶ Rat anterior pituitary cells⁹
- ▶ Transfected COS-7 cells¹⁰
- ▶ Transiently transfected primary rat astrocytes¹¹
- ▶ Rat primary cerebellar granule neurons¹²⁻¹³
- ▶ Murine microglia MG-7 cells¹⁴

Source

- ▶ PDL, synthetic (MW 75-150 kD)
- ▶ PLL, synthetic (MW 30-70 kD)

Quality Control

- ▶ Tested for ability to promote firm attachment of RCG cells
- ▶ Tested and found negative for bacteria and fungi

Storage

4°C to 30°C under dry conditions.

Effect of Corning BioCoat PDL on Cortical Neurons



Mixed culture of cortical neurons and astrocytes cultured on Corning BioCoat PDL cultureware. Neurons are highly branched with very long processes. Astrocytes show similar process elongation.

Effect of Corning BioCoat PDL on RCG Cells



Rat cerebellar granule (RCG) cells cultured on Corning BioCoat PDL cultureware show firm attachment (similar results obtained on PLL).

References

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2. Sugawara, T., et al., PNAS 98(11):6384 (2001).
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5. Hu, L.A., et al., J. Biol. Chem. 275:38659 (2000).
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Corning® BioCoat™ Collagen IV Cultureware

Type IV Collagen is a ubiquitous component in basement membranes and provides the major structural support for this matrix. When the Collagen IV meshwork is assembled, it provides a scaffold for the assembly of other basement membrane components through interactions with laminin, entactin/nidogen, and heparan sulfate proteoglycan. Collagen IV is useful as a substrate for growth of epithelial, endothelial, muscle, and nerve cells. Collagen plays a role in the regulation of cell growth, differentiation and adhesion, as well as tissue formation.

Applications include:

- ▶ Promotion of cell attachment and spreading
- ▶ Cell differentiation and neurite outgrowth
- ▶ Increased proliferation of PC12 cells
- ▶ Studies of effects of collagen IV on cell behavior
- ▶ Cell adhesion assays

Has been used to culture:

- ▶ PC12 cells¹⁻³
- ▶ SH-SY5Y cells¹
- ▶ Human melanoma cells lines SK-MEL-28-N1 and SK-MEL-28⁴
- ▶ Primary murine hepatocytes⁵

Source

Engelbreth-Holm-Swarm (EHS) lathrytic mouse tumor

Quality Control

- ▶ Tested for ability to initiate differentiation (neurite outgrowth) of NG-108 rat glioma/mouse neuroblastoma cells
- ▶ Tested and found negative for bacteria and fungi
- ▶ Collagen IV purity >90% by SDS-PAGE

Storage

2°C to 8°C. Do not freeze.

References

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2. Marchetti, D., et al., Int. J. Cancer 55:692 (1993).
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5. Swift, L.L., et al., J. Biol. Chem. 276(25):22965 (2001).

Effects of Corning BioCoat Collagen IV Cultureware on PC12 Rat Pheochromocytoma Cells



PC12 cells cultured on tissue culture plastic do not attach well and tend to float in clumps in the culture medium.



PC12 cells cultured on Corning BioCoat Collagen IV cultureware show 90% attachment and rapid proliferation.

Corning® BioCoat™ Fibronectin Cultureware

Human Fibronectin (HFN) is a widely distributed glycoprotein that is used as a substrate to promote attachment of cells through its central-binding domain RGD sequence. HFN is a product of most mesenchymal and epithelial cells and is present in both the ECM and plasma. The principal function of HFN appears to be in cellular migration during wound healing and development, regulation of cell growth and differentiation, and haemostasis/thrombosis.

Applications include:

- ▶ Promotion of cell attachment and spreading
- ▶ Rapid expansion of cell populations
- ▶ Serum-free or reduced-serum culture
- ▶ Cell adhesion assays
- ▶ Studies of effects of HFN on cell behavior
- ▶ Improving survival of primary cells in culture

Has been used to culture:

- ▶ 3T3 Preadipocytes¹
- ▶ Transfected 293T and transfected H1299 cells²
- ▶ MCF-10A cells³
- ▶ Primary cord blood mononuclear cells⁴
- ▶ SK-MEL-28 (human melanoma cells)⁵
- ▶ NIH3T3 cells⁶
- ▶ MDA-231 human breast cancer cells⁷

Source

Human plasma

NOTE: Source material tested for hepatitis B antigen and HIV-1 antibody

Quality Control

- ▶ Tested for ability to promote attachment and spreading of BHK-21 hamster kidney cells
- ▶ Tested and found negative for bacteria and fungi
- ▶ Fibronectin purity >90% by SDS-PAGE

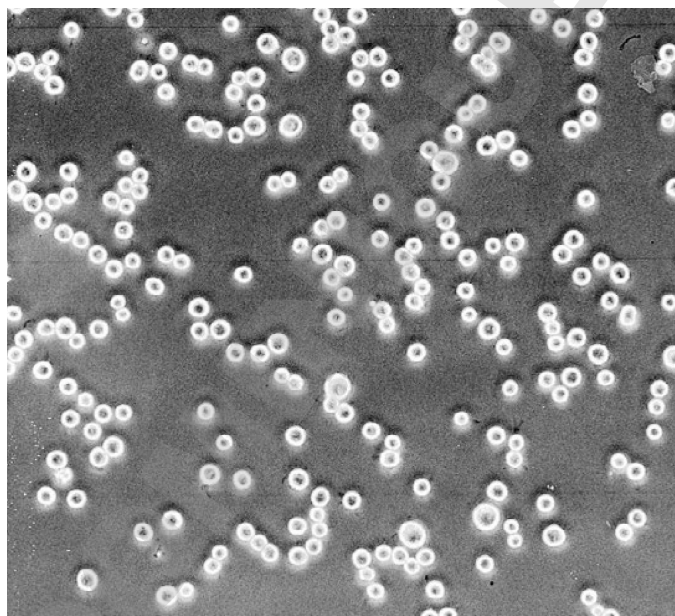
Storage

2°C to 8°C. Do not freeze.

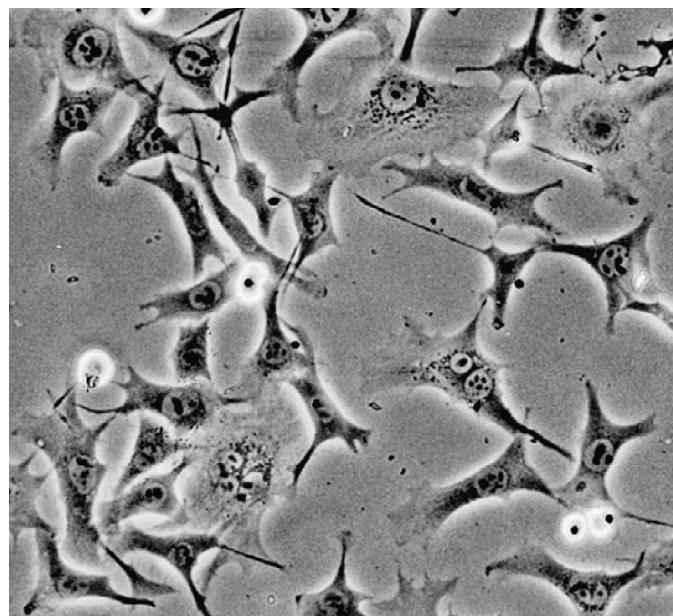
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3. Miller, K.A., et al., *J. Biol. Chem.* 275:8176 (2000).
4. Murohara, T., et al., *J. Clin. Invest.* 105:1527 (2000).
5. Nakano, J., et al., *J. Invest. Derm. Symp. Proc.* 4:173 (1999).
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7. Yoneda, T., et al., *J. Clin. Invest.* 99:2509 (1997).

Effects of Corning BioCoat Fibronectin Cultureware on BHK-21 Cells



BHK-21 fibroblasts cultured on glass cultureslides do not spread.



BHK-21 fibroblasts cultured on Corning BioCoat HFN cultureslides attach and spread within one hour.

Corning® BioCoat™ Laminin Cultureware

Laminin (LM), a major component of basement membranes, is a multifunctional glycoprotein that is used as a substrate to culture and maintain differentiated function of a wide variety of cells. Laminin has been shown in culture to stimulate neurite outgrowth, promote cell attachment, chemotaxis, cell differentiation, and neuronal survival.

Applications include:

- ▶ Promotion of cell attachment and spreading
- ▶ Induction of cell differentiation and neurite outgrowth
- ▶ Increases proliferation of myoblasts¹
- ▶ Studies of effects of laminin on cell behavior
- ▶ Cell adhesion assays

Has been used to culture:

- ▶ SH-SY5Y (human neuroblastoma), Neuro-2A (mouse neuroblastoma), N1-E115 (rat neuroblastoma)²
- ▶ MCF-10A cells^{3,4}
- ▶ SK-MEL-28 cells⁵
- ▶ HVSMC⁶
- ▶ MDA-231 breast cancer cell line⁷

Source

Engelbreth-Holm-Swarm (EHS) mouse tumor

Quality Control

- ▶ Tested for ability to initiate differentiation (neurite outgrowth) of NG-108 rat glioma/mouse neuroblastoma cells
- ▶ Tested and found negative for bacteria and fungi
- ▶ Laminin purity >90% by SDS-PAGE (contains entactin)

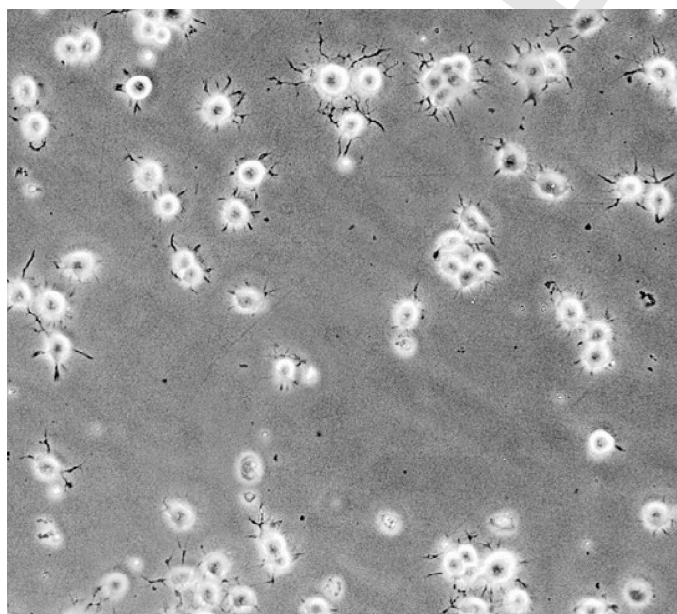
Storage

2°C to 8°C. Do not freeze.

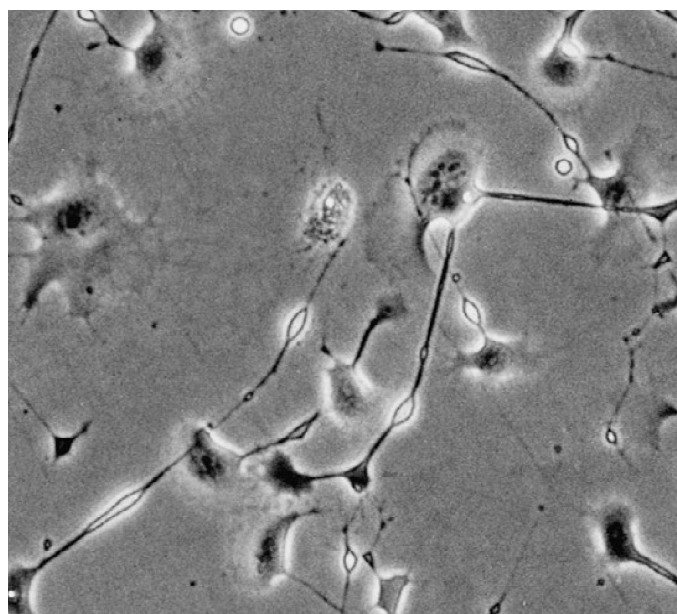
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Effects of Corning BioCoat LM Cultureware on NG-108 Rat Glioma/Mouse Neuroblastoma Cells



NG-108 rat glioma/mouse neuroblastoma cells cultured on tissue culture plastic are loosely adhered and remain rounded.



NG-108 rat glioma/mouse neuroblastoma cells cultured on Corning BioCoat LM cultureware exhibit a spindle-shaped morphology and dendritic processes.

Corning® BioCoat™ Matrigel® Matrix Cultureware

Corning Matrigel basement membrane matrix is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in ECM proteins. Its major component is laminin, followed by collagen IV, heparan sulfate proteoglycans, entactin, and nidogen. Corning Matrigel matrix is effective for the attachment and differentiation of both normal and transformed anchorage-dependent epithelial and other cell types including neurons and oligodendrocytes.

Applications include:

- ▶ Elicitation of tissue-specific cellular morphology and protein production in epithelial cells
- ▶ Differentiation of endothelial, muscle, and neuronal cells
- ▶ Development of three-dimensional matrix model systems

Has been used to culture:

- ▶ Rat hepatocytes¹
- ▶ Primary human hepatocytes²
- ▶ Mouse pituitary gland tissue³
- ▶ Rabbit colonocytes⁴
- ▶ Human urothelial cells⁵
- ▶ Osteopontin (OPN) deficient rat vascular smooth muscle cells⁶

Source

EHS mouse tumor

Formulation

Dulbecco's Modified Eagles' Medium with 50 µg/mL gentamycin. Corning Matrigel matrix is compatible with all culture media.

Quality Control

- ▶ Tested for ability to promote neurite outgrowth from chick dorsal root ganglia in the absence of NGF
- ▶ Tested and found negative for bacteria and fungi

Storage and Stability

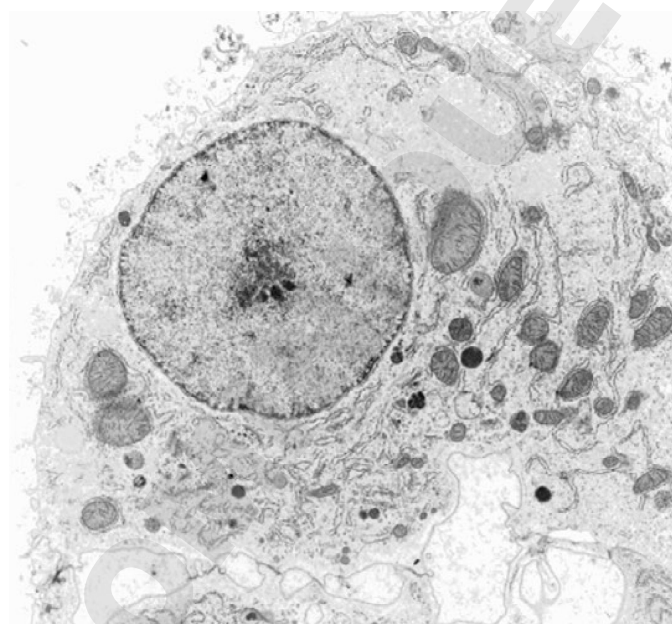
- ▶ -20°C. Keep frozen until use.
- ▶ Thin layer cultureware stable at 2°C to 8°C.

References

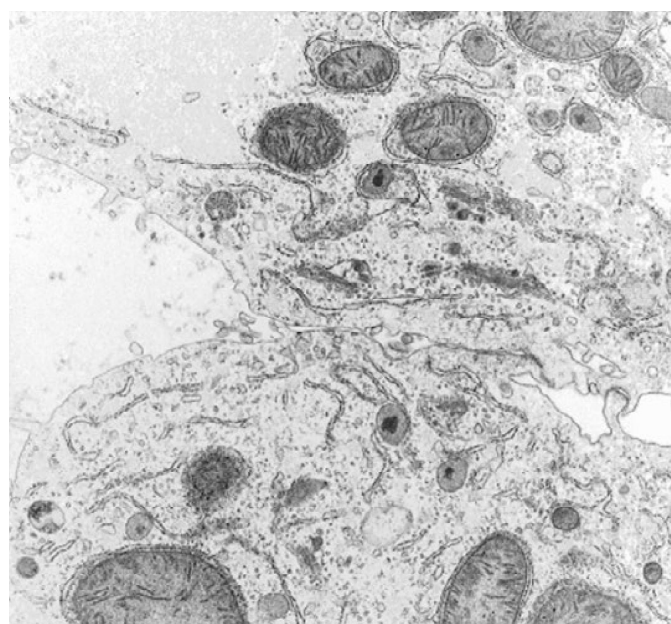
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Rat Hepatocytes Cultured in the Corning BioCoat Hepatocyte Differentiation Environment

Transmission Electron Micrograph of thin sections shows similar intracellular and intercellular structures, indicative of healthy differentiated hepatocytes, in 4-week-old cultures.



Intracellular structures active nucleus (5,000X). Numerous mitochondria with calcium deposits; rough ER stacks near cell surfaces and surrounding mitochondria; glycogen stores; golgi, ribosomal rosette, lipid droplets.



Intercellular structures (8,600X). Frequent interdigitation of adjoining cells; gap and tight junctions; intercellular lumens with microvilli, characteristic of bile canaliculi.

Corning® BioCoat™ Poly-D-Lysine/Laminin, Poly-L-Ornithine/Laminin, and Laminin/Fibronectin Cultureware

For some applications, the use of a combination of ECM proteins, such as Laminin (LM) and Fibronectin (HFN) or LM and attachment factors such as Poly-D-Lysine (PDL) or Poly-L-Ornithine (PLO) has been shown superior to the use of either alone.

Corning BioCoat PDL/LM and PLO/LM Cultureware is suitable for culturing many different types of Peripheral Nervous System (PNS) and Central Nervous System (CNS) networks and is useful for promoting neural cell attachment and differentiation. Corning BioCoat LM/HFN Cultureware provides an *in vitro* environment that promotes cell attachment and extensive process formation.

Applications include:

- ▶ Enhancement of neuronal cell attachment to plastic and glass
- ▶ Promotion of neurite outgrowth
- ▶ Culture of glial cells as a feeder layer for neurons
- ▶ Construction of neural cell model systems to study CNS function, development, and diseases

Corning BioCoat PDL/LM has been used to culture:

- ▶ SH-SY5Y (human neuroblastoma), Neuro-2A (mouse neuroblastoma), N1-E115 (rat neuroblastoma)¹
- ▶ Primary rat hippocampus²
- ▶ Murine T11-L3 dorsal root ganglion neurons (DRGNs)^{3,4}
- ▶ Transfected PC12 cells⁴
- ▶ MCF-10A cells⁵
- ▶ Rat primary DRGNs⁶

Source

- ▶ PDL, synthetic (MW 75-150 kD)
- ▶ PLO, synthetic (MW 30-70 kD)
- ▶ Laminin, EHS mouse tumor
- ▶ Fibronectin, human plasma
- ▶ **NOTE:** Source material tested for hepatitis B antigen and HIV-1 antibody.

Quality Control

- ▶ PDL/LM and PLO/LM tested for ability to initiate differentiation (neurite outgrowth) of NG-108 rat glioma/mouse neuroblastoma cells
- ▶ LM/HFN tested for receptor agonist induced changes in intracellular calcium using Fluo-3A (a long wavelength fluorescent calcium indicator) in primary rat cortical neuron enriched cultures^{7,8}
- ▶ Tested and found negative for bacteria and fungi

Storage

2° to 8°C. Do not freeze.

Effects of Corning BioCoat Laminin/Fibronectin on Astrocytes



Neurotransmitter induced calcium waves in astrocytes plated on Corning BioCoat Laminin/Fibronectin cultureware.

References

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8. Cornell-Bell, A.H., et al., *Science*, 247:470 (1990).

Corning® BioCoat™ T-Cell Activation Plates

Plate-bound antibodies against the T-cell receptor complex have been used to induce activation of T-cells from a variety of species without the help of accessory cells. Corning BioCoat T-Cell activation plates are precoated with high quality BD Pharmingen™ CD3 antibodies. Available for use with mouse or human T-cells, Corning BioCoat T-Cell activation plates offer lot-to-lot consistency and come individually packaged with lids for ease of use.

Applications include:

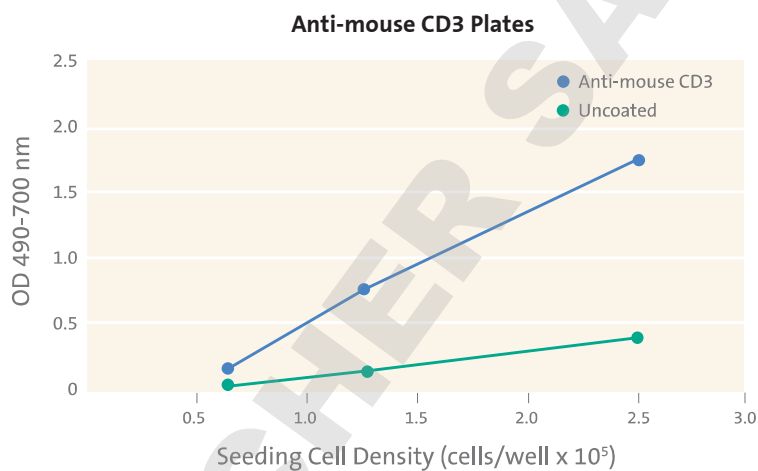
- ▶ T-Cell activation
- ▶ Cytokine production
- ▶ Cytokine mRNA quantitation
- ▶ Co-stimulation
- ▶ Studies of drug effects on T-cell function

Quality Control

- ▶ Tested for ability to proliferate mouse splenocytes or human PBMCs
- ▶ Tested and found negative for bacteria and fungi

Storage

2°C to 8°C. Do not freeze.



48-hour incubation with mouse splenocytes on mouse anti-CD3 plates followed by a 4-hour MTS assay.



48-hour incubation with human PBMCs followed by cell proliferation assay using BrdU (6-hour labeling).

Corning® BioCoat™ Variety Pack Cultureware Products and Vented Caps for Corning BioCoat Flasks

Corning BioCoat Variety Pack

Corning BioCoat Variety Packs each contain 6-well multiwell plates or cultureslides with a selection of different extracellular matrix proteins and attachment factors.

Applications include:

- ▶ Determination of optimal substrate for growth or differentiation of particular cell types
- ▶ Studies of effects of various ECM components on cell behavior
- ▶ Cell adhesion assays

Quality Control

- ▶ Tested for ability to promote cell growth or differentiation (cell type used is indicated for each individual Corning BioCoat cultureware product)
- ▶ Tested and found negative for bacteria and fungi

Storage

2°C to 8°C. Do not freeze.

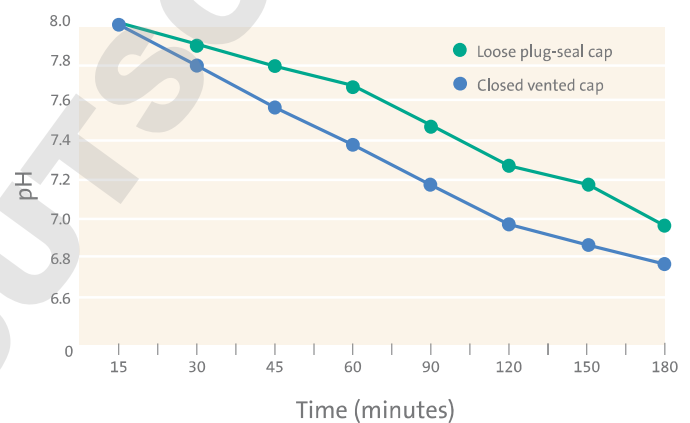
Vented Caps for Corning BioCoat Flasks

Vented caps are available for use with Corning BioCoat flasks. The vented caps are made from polyethylene and contain a 0.2 µm membrane vent that allows consistent gas exchange but prevents passage of bacteria and fungi. The special design reduces the risk of contamination associated with standard cell culture open incubation.



Storage

Store at ambient temperature.



pH equilibration using vented caps after flasks are placed in an incubator (175 cm² flasks, 5% CO₂ incubator).

Corning® BioCoat™ Collagen and Poly-D-Lysine 96-, 384-, and 1536-well Microplates for High Throughput Screening

Application Focus: Analysis of Transfected Cell Lines

Corning BioCoat products coated with extracellular matrix proteins (ECM) and cell attachment factors are widely used to promote cell attachment, proliferation, and differentiation. For high throughput screening during the drug discovery process, cell-based assays are used to identify drug candidates that exhibit a desired effect upon target function.

To provide an appropriate biological background, functional assays can be performed using transfected cell lines that express a wild-type or mutated gene of interest. Many transfected cell lines are susceptible to reduced adherence when 96-, 384-, or 1536-well assay plates are subjected to standard wash protocols during high throughput sample processing. Although vigorous washing is essential for reducing background noise, this treatment can result in sample loss due to disruption of the cell monolayer. Therefore, the acquisition of reliable data can be dramatically compromised when transfected cells exhibit weak attachment to the culture substrate. In contrast, transfected cell lines adhere strongly to assay plates that are coated with a cell attachment substrate.

A number of cell types are used for high throughput transfection analyses. The human embryonic kidney cell line (HEK-293) is a common choice for stable transfections. Although these cells are useful for expressing a wide variety of transfected genes, HEK-293 cells are especially susceptible to reduced adherence on standard assay plates during high throughput sample processing. However, strong adherence has been observed when transfected HEK-293 cells are cultured on BioCoat Poly-D-Lysine (PDL) (Figure 1), Poly-L-Lysine (PLL), or Collagen I cultureware. A number of unique formulations have been shown to dramatically improve cell adherence during high throughput processing of samples cultured in both serum-free and serum-containing media. BioCoat PDL 384-well black/clear and white/clear plates have been reformulated to further enhance their performance in a number of specialized assays that demand superior cell attachment. Optimized BioCoat PDL 384-well black/clear plates exhibit superior cell attachment and lower CVs over all competitors.

BioCoat cultureware is also effective for high throughput applications that utilize neuronal cell lines. For example, transfected human astrocytoma cells (1321N1) and PC12 cells have been cultured on BioCoat PDL and Collagen I, respectively. Also, a variety of BioCoat products are available that support neurite outgrowth and neurotransmitter receptor function.

Corning BioCoat 96-, 384-, and 1536-well Microplates



Corning offers a wide selection of microplates for cell-based fluorescence, luminescence, colorimetric, and radiometric assays.

High throughput studies of a variety of cell types (transfected and untransfected) have also been carried out using Corning BioCoat Fibronectin (HFN), Collagen IV, and PDL/Laminin (LM). Overall, BioCoat cultureware has been found to dramatically improve cell adherence during high throughput sample processing. In this regard, the appropriate culture substrate will contribute to the reliability of high throughput transfection analyses by providing optimal conditions for cell attachment and growth.

Applications

- ▶ Reporter gene assays
- ▶ Ion channel activity
- ▶ Receptor binding
- ▶ Cytotoxicity assays
- ▶ Apoptosis assays
- ▶ Cell adhesion kinetics
- ▶ Cell proliferation assays
- ▶ Calcium flux assays

Culture Substrates for Transfected Cells

Cell Attachment Substrate	Cell Type
Corning BioCoat™ Poly-D-Lysine (PDL)	HEK-293 293 EBNA Cardiomyocyte Human astrocytoma (1321N1) Mouse pituitary (AtT-20) Pancreatic islet (RIN-m) COS-7
Corning BioCoat Poly-L-Lysine (PLL)	HEK-293 PC12
Corning BioCoat Collagen I	CHO HEK-293 PC12 SR-3T3
Corning BioCoat Fibronectin	Pancreatic tumor (AR42J) COS-7
Corning Cell-Tak™ Cell and Tissue Adhesive	HEK-293 L9 mouse fibroblasts

Quality Control

- ▶ Poly-D-Lysine and Collagen I: Tested for the attribute of cell attachment and growth utilizing an attachment-dependent mammalian cell line in a serum-free media. Plate percent CVs are measured to ensure consistent coating.
- ▶ Tested and found negative for bacteria and fungi

Storage

4°C to 30°C under dry conditions.

Improved HEK-293 Cell Adhesion Post-transfection with Corning BioCoat Assay Plates



Figure 1. EcoPack 2-293 cells in serum-free media cultured on Corning BioCoat PDL 384-well black/clear plates and TC-treated black/clear plates. Results are pre- and post-washing on a Skatron washer (Molecular Devices). Cells exhibit poor adhesion to TC-treated plates. In contrast, these cells exhibit strong attachment following vigorous washing steps on the Corning BioCoat PDL plates. EcoPack 2-293 is a transformed HEK-293 cell line (Clontech).

Corning® Pre-coated Permeable Supports

Permeable supports, also known as cell culture inserts, contain a microporous membrane that allows cells to be fed both apically and basolaterally, which mimics the *in vivo* cell environment. These culture systems have been found to support optimal cell differentiation and functionality *in vitro* and are used in a wide range of applications. Corning BioCoat™ cell culture inserts are pre-coated with extracellular matrix (ECM) proteins to provide cells with an enriched growth environment, which further supports cell functionality for applications requiring a protein-coated cell surface, such as cell differentiation, migration and invasion assays.

Various ECM coatings are available, including Corning Matrigel® matrix, Fibronectin, and Collagen. Coated inserts are also available with a variety of membrane types (including PET and Corning FluoroBlok™), pore sizes (0.4 to 8.0 µm), and device formats (individual and HTS). BioCoat insert systems are available in 24- and 96-well multiwell formats that have been optimized for specific assays, including angiogenesis, tumor cell invasion, Caco-2, and intestinal epithelium differentiation.

For example, cells grown on permeable supports coated with Fibrillar Collagen I can establish the barrier function of intestinal epithelial cell monolayers (Caco-2). In addition, inserts coated with Matrigel matrix are frequently cited for *in vitro* cell invasion assays that are often used in cancer research.

See the *Corning Permeable Supports Selection Guide (CLS-CC-027)* for a full and up-to-date listing of our pre-coated cell culture insert products.



Scanning electron micrograph of two human fibrosarcoma cells (HT-1080 cells), having digested the Corning Matrigel matrix occluding the membrane and migrating through an 8.0 µm pore of the PET membrane.

Application Areas

- ▶ Cell differentiation
- ▶ Co-culture
- ▶ Cell migration and invasion
- ▶ Angiogenesis
- ▶ Transport and permeability
- ▶ Toxicity testing



Corning BioCoat individual cell culture inserts



Corning BioCoat multiwell insert systems



Corning BioCoat xxxx

Ordering Information

Corning® BioCoat™ Collagen I Cultureware

Multiwell and Assay Plates

Cat. No.	Description	Qty/Pk
354400	6-well	5
356400	6-well	50
354500	12-well	5
356500	12-well	50
354408	24-well	5
356408	24-well	50
354505	48-well	5
356505	48-well	50
354407	96-well clear	5
356407	96-well clear	50
356698	96-well clear	80
354649	96-well black/clear	5
356649	96-well black/clear	50
356700	96-well black/clear	80
4582	96-well half area black/clear glass bottom	10
354650	96-well white/clear	5
356650	96-well white/clear	50
356701	96-well white/clear	80
354519	96-well white	5
356519	96-well white	50
356699	96-well white	80
354666	384-well clear	5
356666	384-well clear	50
356704	384-well clear*	80
354667	384-well black/clear	5
356667	384-well black/clear	50
356705	384-well black/clear	80
4583	384-well black/clear glass bottom	10
354397	384-well small-volume black/clear	5
356397	384-well small-volume black/clear	50
354664	384-well white/clear	5
356664	384-well white/clear	50
356702	384-well white/clear*	80
354665	384-well white	5
356665	384-well white	50
356703	384-well white*	80

Culture Dishes

354456	35 mm	20
356456	35 mm	100
354401	60 mm	20
356401	60 mm	100
354450	100 mm	10
356450	100 mm	40
354551	150 mm	5

Flasks

Cat. No.	Description	Qty/Pk
354484	25 cm ² , vented-cap cap [†]	10
356484	25 cm ² , vented-cap cap [†]	50
354485	75 cm ² , vented-cap cap [†]	5
356485	75 cm ² , vented-cap cap [†]	50
354486	150 cm ² , vented-cap cap	5
356486	150 cm ² , vented-cap cap	40
354487	175 cm ² , vented-cap cap	5
356487	175 cm ² , vented-cap cap	40

Coverslips

354089	22 mm round No.1 German glass	60
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CultureSlides

354557	4-well	12
354630	8-well	12

Corning BioCoat Poly-D-Lysine Cultureware

Multiwell and Assay Plates

354413	6-well	5
356413	6-well	50
354470	12-well	5
356470	12-well	50
354414	24-well	5
356414	24-well	50
354509	48-well	5
356509	48-well	50
354461	96-well clear	5
356461	96-well clear	50
356690	96-well clear	80
354640	96-well black/clear	5
356640	96-well black/clear	50
356692	96-well black/clear	80
4586	96-well half area black/clear glass bottom	10
354651	96-well white/clear	5
356651	96-well white/clear	50
356693	96-well white/clear	80
354620	96-well white	5
356620	96-well white	50
356691	96-well white	80
354662	384-well clear	5
356662	384-well clear	50
356696	384-well clear*	80
354663	384-well black/clear	5
356663	384-well black/clear	50
356697	384-well black/clear	80
4587	384-well black/clear glass bottom	10
354396	384-well small-volume black/clear	5
356396	384-well small-volume black/clear	50
354660	384-well white/clear	5

Multiwell and Assay Plates (Continued)

Cat. No.	Description	Qty/Pk
356660	384-well white/clear	50
356694	384-well white/clear*	80
354661	384-well white	5
356661	384-well white	50
356695	384-well white*	80
354022	1536-well black/clear	5
356022	1536-well black/clear	50

Culture Dishes

354467	35 mm	20
356467	35 mm	100
354468	60 mm	20
356468	60 mm	100
354469	100 mm	10
356469	100 mm	40
354550	150 mm	5

Flasks

354536	25 cm ² , vented-cap [†]	10
356536	25 cm ² , vented-cap [†]	50
354537	75 cm ² , vented-cap [†]	5
356537	75 cm ² , vented-cap [†]	50
354538	150 cm ² , vented-cap	5
356538	150 cm ² , vented-cap	40
354539	175 cm ² , vented-cap	5
356539	175 cm ² , vented-cap	40

Coverslips

354086	12 mm round No.1 German glass	80
354077	35 mm Coverslip-bottom dishes	20

CultureSlides

Cat. No.	Description	Qty/Pk
354577	4-well	12
354632	8-well	12

Corning BioCoat Poly-L-Lysine Cultureware

Multiwell and Assay Plates

354515	6-well	5
356515	6-well	50
354516	96-well clear	5
356516	96-well clear	50

Culture Dishes

354518	35 mm	20
356518	35 mm	100
354517	60 mm	20
356517	60 mm	100

Coverslips

354085	12 mm round No.1 German glass	60
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[†]Corning BioCoat 25 cm² flasks are 70 mL canted neck; Corning BioCoat 75 cm² flasks are 250 mL canted neck.

*Please call for shipping schedules on large orders.

Ordering Information (Continued)

Corning® BioCoat™ Gelatin Cultureware

Multiwell and Assay Plates

Cat. No.	Description	Qty/Pk
354652	6-well	5
356652	6-well	50
354689	96-well	5
356689	96-well	50

Culture Dishes

Cat. No.	Description	Qty/Pk
354653	100 mm	10
356653	100 mm	40

Flasks

354488	75 cm ² , vented-cap cap [†]	5
356488	75 cm ² , vented-cap cap [†]	50

Corning BioCoat Collagen IV Cultureware

Multiwell and Assay Plates

354428	6-well	5
354430	24-well	5
354429	96-well	5

Culture Dishes

354459	35 mm	20
354416	60 mm	20
354453	100 mm	10

Flasks

354534	25 cm ² , plug-seal cap [†]	10
354523	75 cm ² , plug-seal cap [†]	10
354528	175 cm ² , plug-seal cap	5

Corning BioCoat Laminin Cultureware

Multiwell and Assay Plates

354404	6-well	5
354502	12-well	5
354412	24-well	5
354507	48-well	5
354410	96-well	5

Culture Dishes

354458	35 mm	20
354405	60 mm	20
354452	100 mm	10
354553	150 mm	5

Flasks

354533	25 cm ² , plug-seal cap [†]	10
354522	75 cm ² , plug-seal cap [†]	10

Corning BioCoat Fibronectin Cultureware

Multiwell and Assay Plates

Cat. No.	Description	Qty/Pk
354402	6-well	5
354501	12-well	5
354411	24-well	5
354506	48-well	5
354409	96-well	5

4584	96-well half area black/clear glass bottom	10
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4585	384-well black/clear glass bottom	10
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Culture Dishes

354457	35 mm	20
354403	60 mm	20
354451	100 mm	10
354552	150 mm	5

Flasks

354532	25 cm ² , plug-seal cap [†]	10
354521	75 cm ² , plug-seal cap [†]	10
354646	150 cm ² , plug-seal cap	5
354526	175 cm ² , plug-seal cap	5

Coverslips

354088	22 mm round No. 1 German glass	60
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CultureSlides

354559	4-well	12
354631	8-well	12

Corning BioCoat Laminin/Fibronectin Cultureware

Assay Plates

354670	96-well	5
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Corning BioCoat Matrigel® Matrix Cultureware

Multiwell Plates

354432	6-well	2
354503	12-well	2
354433	24-well	2
354508	48-well	2

Culture Dishes

354460	35 mm	8
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Corning BioCoat Matrigel Matrix Cultureware — Thin Layer

Multiwell and Assay Plates

354603	6-well	5
354605	24-well	5
354607	96-well	5

Culture Dishes

354602	35 mm	20
354601	60 mm	20
354600	100 mm	10

Corning BioCoat Matrigel Matrix Cultureware for Hepatocytes

Multiwell Plates

Cat. No.	Description	Qty/Pk
354510	6-well	5

Corning BioCoat GFR Matrigel Matrix Cultureware for Smooth Muscle Cells

Multiwell Plates

354635	24-well	5
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Corning BioCoat Poly-D-Lysine/Laminin Cultureware

Multiwell and Assay Plates

354595	6-well	5
354619	24-well	5
354596	96-well	5

Culture Dishes

354455	100 mm	10
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Coverslips

354087	12 mm round No.1 German glass	80
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CultureSlides

354688	8-well	12
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Corning BioCoat™ Poly-L Ornithine/Laminin Cultureware

Multiwell and Assay Plates

354658	6-well	5
354659	24-well	5
354657	96-well	5

Corning BioCoat T-Cell Activation Plates

Assay Plates

354720	Mouse Anti-CD3 96-well clear	5
354725	Human Anti-CD3 96-well clear	5
354730	Uncoated Control	5

Vented Caps for Corning BioCoat Flasks

354637	25 cm ²	100
354638	75 cm ²	100
354639	175 cm ²	50

Corning BioCoat Variety Pack Cultureware

Multiwell Plates

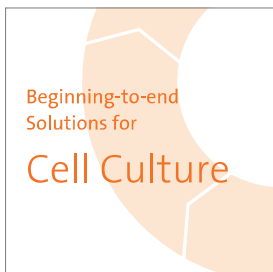
354417	6-well	5
<i>Includes: Collagen I, Fibronectin, Laminin, Poly-D-Lysine, and Falcon® plates</i>		
354431	6-well	5
<i>Includes: Collagen I, Collagen IV, Fibronectin, Laminin, and Poly-D-Lysine plates</i>		

CultureSlides

354656	8-well	12
<i>Includes: Collagen I, Fibronectin, Poly-D-Lysine, and Falcon cultureslides</i>		

[†]Corning BioCoat 25 cm² flasks are 70 mL canted neck; Corning BioCoat 75 cm² flasks are 250 mL canted neck.

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At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

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