

BD BioCoat™ Cellware

BD Biosciences

Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen



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About the cover:

Computer colorization of, from top to bottom:

- ▶ Rat cerebellar granule (RCG) cells cultured on BD BioCoat™ PDL (page 6)
- ▶ Human umbilical vein endothelial cells (HUVECs) grown for seven days on BD BioCoat™ Gelatin 6-well Multiwell Plates (page 5)
- ▶ BHK-21 fibroblasts cultured on BD BioCoat™ Fibronectin CultureSlides (page 9)
- ▶ Neurotransmitter induced calcium waves in astrocytes plated on BD BioCoat™ Laminin/Fibronectin (page 13)

Introduction



BD BioCoat™ Cellware

BD BioCoat™ Cellware 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.

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 injury.¹

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.

BD BioCoat Cellware 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 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

Get the BD BioCoat™ Advantages

Ready to Use Convenience

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

Quality Assurance Testing

Each lot of BD BioCoat Cellware is thoroughly tested for bioactivity and guaranteed to perform as claimed so you can use with complete confidence.

Reliable Performance

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

Lot-to-Lot Consistency

BD Biosciences prides itself on maintaining highly controlled ISO 9001 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, BD BioCoat helps optimize conditions for attachment, growth or differentiation for your cell type.

Readily Available

BD BioCoat Cellware is available from stock for immediate shipments. Our service options include standing order management and lot number reservations.

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

BD BioCoat™ Cellware Vessel Options

The first company to produce sterile, disposable labware, BD Biosciences is a world leader in providing researchers with top-quality cell culture products. BD Falcon™ Cultureware is treated using a unique vacuum gas plasma process resulting in a pure and consistent tissue culture surface. BD BioCoat™ Cellware is manufactured using BD Falcon products to ensure consistent, reliable results. Trust BD Biosciences, the first name in cell culture.

BD BioCoat™...

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



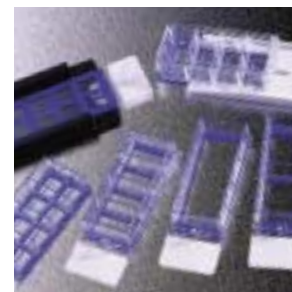
...**Multiwell Plates** are manufactured using a crystal-grade polystyrene and feature a patented labyrinth lid, condensation rings and deep well design to control contamination while reducing evaporation and minimizing edge effects.



...**Dishes** are exceptionally flat for distortion-free optics and feature stacking rings for better stacking. Easy Grip design ensures safe 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 Number 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 style dishes with a coverslip bottom that is easy to use and facilitates preparation of cells for microscopic analysis. The coverslip floor is Number 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.



For BD BioCoat™ Cell Culture Inserts and Assay Systems, please see our general BD Falcon™/BD BioCoat™ catalog.

BD BioCoat™ Manufacturing Facilities



BD Biosciences has a highly controlled manufacturing environment for BD BioCoat™ Collagen I, Gelatin and Poly-Lysine products in its ISO 9001 certified plant in Plymouth, England. ISO certification verifies that the facility meets international quality standards and that BD Biosciences provides assurance to customers that it is totally committed to delivering superior quality and product improvements.

The state-of-the-art cleanroom suite was designed and constructed to meet US, EU and British standards.* All BD BioCoat products are produced under aseptic conditions to minimize the risk of product contamination from bacteria, fungi and particulates.

BD Biosciences also has a highly controlled environment for the BD BioCoat products manufactured in its ISO 9001 certified plant in Bedford, Massachusetts. The cleanroom suite, designed to meet

US standards, ensures that all BD BioCoat products are produced under aseptic conditions.

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

Proprietary formulation and manufacturing techniques allow BD Biosciences to produce room temperature Collagen I, Gelatin and Poly-Lysine Cellware. 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° to 50°C.

*US Federal Standard 209E Class M3.5, British Standard 5295 Class E, EU Guide to GMP for medicinal products (Vol. 4) Class A/B

BD BioCoat™ Collagen I Cellware

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 types.¹

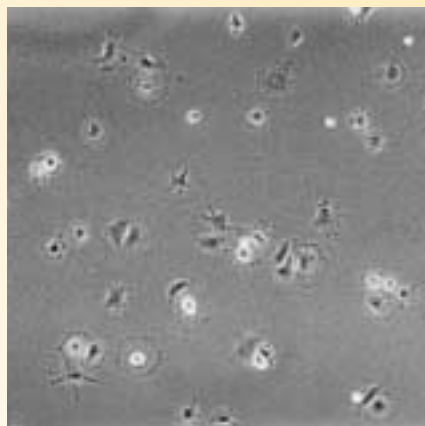
BD BioCoat™ Collagen I Cellware applications include:

- ▶ 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

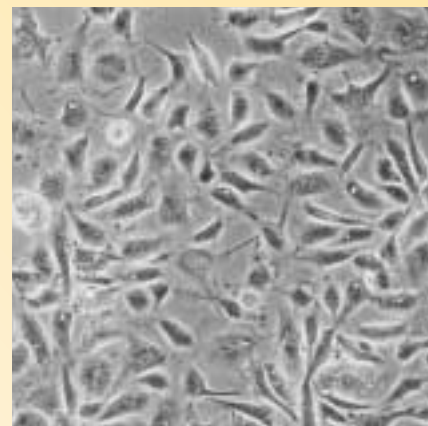
BD BioCoat™ Collagen I has been used to culture:

- ▶ 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

Effects of BD BioCoat™ Collagen I Cellware 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 BD BioCoat Endothelial Cell Growth Environment (Collagen I Cellware) form a confluent monolayer and show numerous mitotic cells.

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 and Stability:

Cellware stable for at least six months from date of shipment when stored at 4° to 30°C under dry conditions. Coverslips and CultureSlides stable for at least three months from date of shipment when stored at 2° to 8°C.

References

1. Kleinman, H.K., et al., *Analytical Biochemistry* **166**:1 (1987).
2. Bjorkegren, J., et al., *J. Biol. Chem.* (Accepted for pub July 31, 2001).
3. Flaherty, P., et al., *BD Tech Bulletin* (1996).
4. Ivankovic-Dikic, I., et al., *Nat. Cell. Biol.* **2**:574-581 (2000).
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6. Nakano, J., et al., *J. Invest. Dermatology Symposium Proc.* **4**(2):173 (1999).
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9. Smith, J.S., et al., *J. Neurosci.* **21**(4):1096 (2001).
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BD BioCoat™ Gelatin Cellware

BD BioCoat™ Gelatin Cellware provides an attachment and growth promoting substrate for the culture of a variety of cell types. Gelatin is used commonly 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.

BD BioCoat™ Gelatin Cellware applications include:

- ▶ Promotion of cell attachment and spreading of:
 - Vascular endothelial cells, i.e. BME¹, BAEC²
 - Embryonic stem (ES) cells³
 - C2C12 myoblasts⁴ and MM14 myoblasts⁵
- ▶ Culture of normal and transfected F9 teratocarcinoma cells for gene expression studies⁶
- ▶ Culture of HUVEC for E-Selectin⁷ expression and VEGF induction⁸

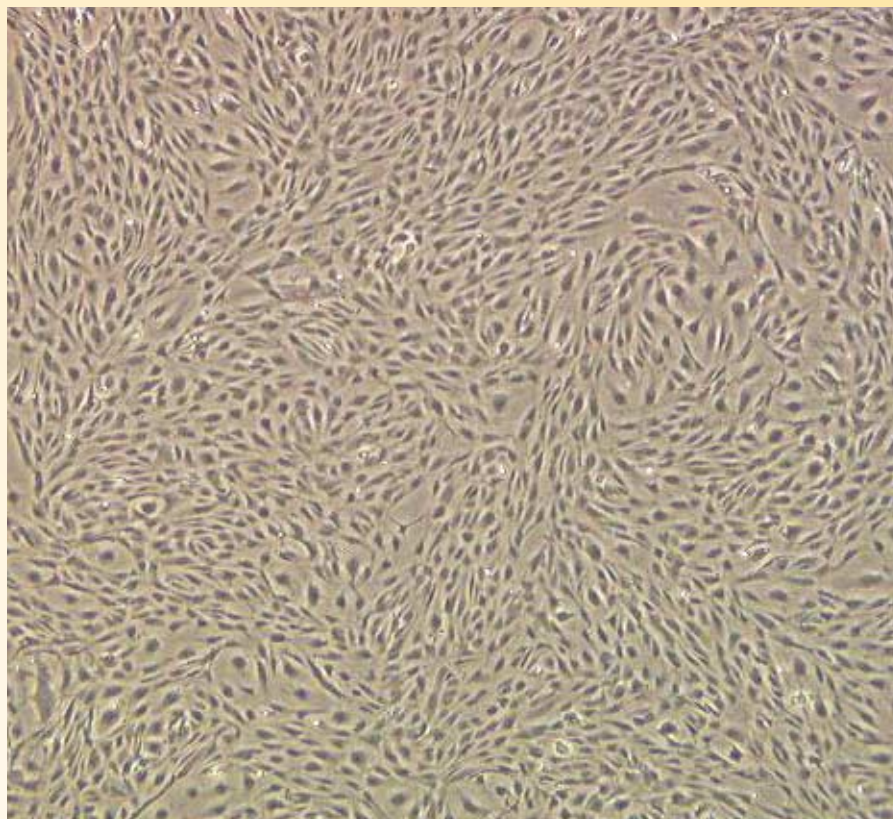
Source:

Gelatin, porcine

Quality Control:

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

Effect of BD BioCoat Gelatin Cellware of HUVEC



Human umbilical vein endothelial cells (HUVECs) grown for seven days on BD BioCoat Gelatin 6-well Multiwell Plates seeded at a density of 2×10^4 in the presence of E-STIM™ Endothelial Cell Culture Medium (440x).

Storage and Stability:

Stable for at least six months from date of shipment when stored at 4° to 30°C under dry conditions.

References

1. Zimrin, A.B., et al., J. Biol. Chem. **271(51)**:32499 (1996).
2. Gou, D., et al., J. Biol. Chem. **270(12)**:6729 (1995).
3. Ernst, M., et al., J. Biol. Chem. **271(47)**:30136 (1996).
4. Stuart, C.E., et al., J. Biol. Chem. **271(19)**:11330 (1996).
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7. Read, et al., J. Biol. Chem. **272(5)**:2753 (1997).
8. Gitay-Goren, et al., J. Biol. Chem. **271(10)**:5519 (1996).

BD BioCoat™ Poly-Lysine Cellware

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.

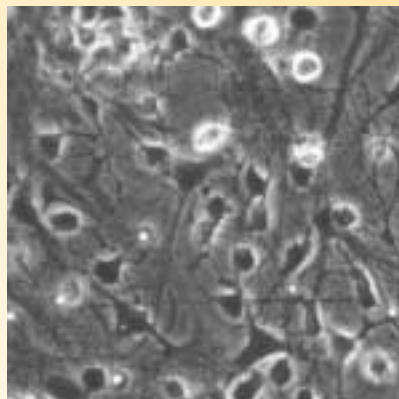
BD BioCoat™ Poly-Lysine Cellware applications include:

- ▶ 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

BD BioCoat™ Poly-Lysine has been used to culture:

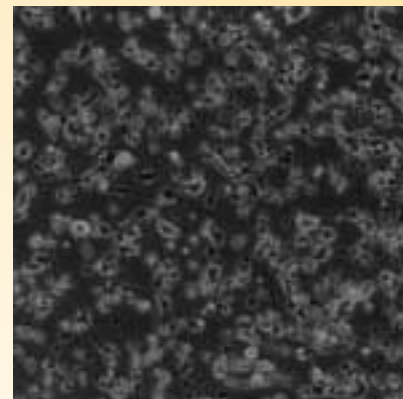
- ▶ Primary mouse brain capillaries¹
- ▶ HEK-293 cells²⁻⁵
- ▶ MDA-231 breast cancer cells⁶
- ▶ Mouse cerebellar granule neurons⁷
- ▶ Transfected rat 1 cells⁸
- ▶ Rat anterior pituitary cells⁹
- ▶ Transfected COS-7 cells¹⁰
- ▶ Transiently transfected primary rat astrocytes¹¹
- ▶ Rat primary cerebellar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

Effect of BD BioCoat PDL on Cortical Neurons



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

Effect of BD BioCoat PDL on RCG Cells



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

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 and Stability:

Cellware stable for at least six months from date of shipment when stored at 4° to 30°C under dry conditions. Coverslips and CultureSlides stable for at least three months from date of shipment when stored at 2° to 8°C.

References

1. Santambrogio, L., et al., PNAS **98(11)**:6295 (2001).
2. Sugawara, T., et al., PNAS **98(11)**:6384 (2001).
3. Bdeir, K., et al., J. Biol. Chem. **275**:28532 (2000).
4. Fitzgerald, L.W., et al., J. Neurochemistry **72(5)**:2127 (1991).
5. Hu, L.A., et al., J. Biol. Chem. **275**:38659 (2000).
6. Yoneda, T., et al., J. Clin. Invest. **99(10)**:2509 (1997).
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8. Bertin, J., et al., J. Biol. Chem. **276(15)**:11877 (2001).
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10. Kirsch, K.H., et al., PNAS **96(11)**:6211 (1999).
11. Little, E.B., et al., PNAS **98(5)**:2238 (2001).
12. Segal, J.A., et al., J. Neurochemistry **74(1)**:60 (2000).
13. Wood, M.W., et al., J. Neurochemistry **74(5)**:2033 (2000).
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BD BioCoat™ Collagen IV Cellware

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.

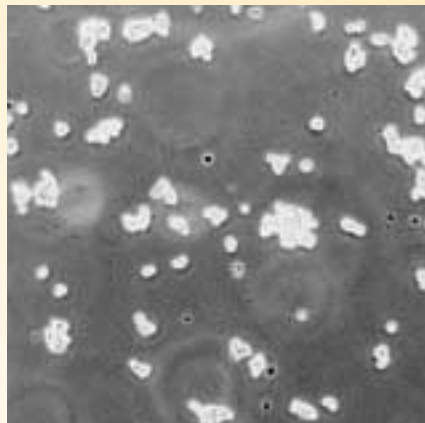
BD BioCoat™ Collagen IV Cellware applications include:

- ▶ 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

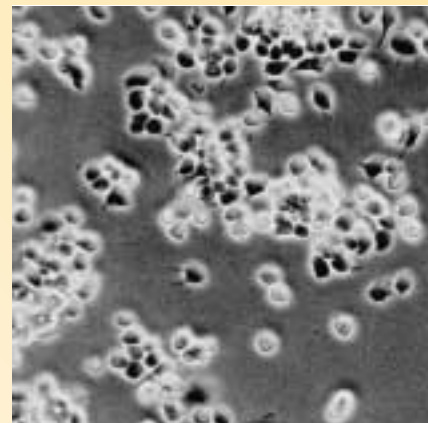
BD BioCoat™ Collagen IV Cellware has been used to culture:

- ▶ PC12 cells¹⁻³
- ▶ SH-SY5Y cells⁴
- ▶ Human melanoma cells lines SK-MEL-28-N1 and SK-MEL-28⁵
- ▶ Primary murine hepatocytes⁶

Effects of BD BioCoat Collagen IV Cellware 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 BD BioCoat Collagen IV Cellware show 90% attachment and rapid proliferation.

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 and Stability:

Stable for at least three months from date of shipment when stored at 2° to 8°C.

References

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2. Marchetti, D., et al., Int. J. Cancer **55**:692 (1993).
3. Muda, M., et al., J. Biol. Chem. **271**:4319 (1996).
4. Ivankovic-Dikic, I., et al., Nat. Cell. Biol. **2**:574 (2000).
5. Nakano, J., et al., J. Invest. Derm. Symp. Proc. **4**(2):173 (1999).
6. Swift, L.L., et al., J. Biol. Chem. **276**(25):22965 (2001).

BD BioCoat™ EHS Matrix Cellware

EHS Matrix is a natural ECM that is synthesized, secreted and formed *in vitro* by a continuous cell line established from primary tissue of the EHS tumor. The ECM secreted by these cells is similar to that which surrounds the *in vivo* EHS tumor. This *in vitro* ECM is composed of laminin, collagen IV and other components which have not as yet been fully characterized.¹

BD BioCoat™ EHS Matrix Cellware has been used to culture:

- ▶ Neural cell attachment and differentiation
- ▶ Transduction of neuroblastomas²
- ▶ Culture of fish hepatocytes
- ▶ Amniocyte attachment and culture
- ▶ Primary tumor-cell culture
- ▶ Dunning R-3327 rat prostatic adenocarcinoma cells³

Source:

Monolayers of a cell line established from the EHS mouse tumor

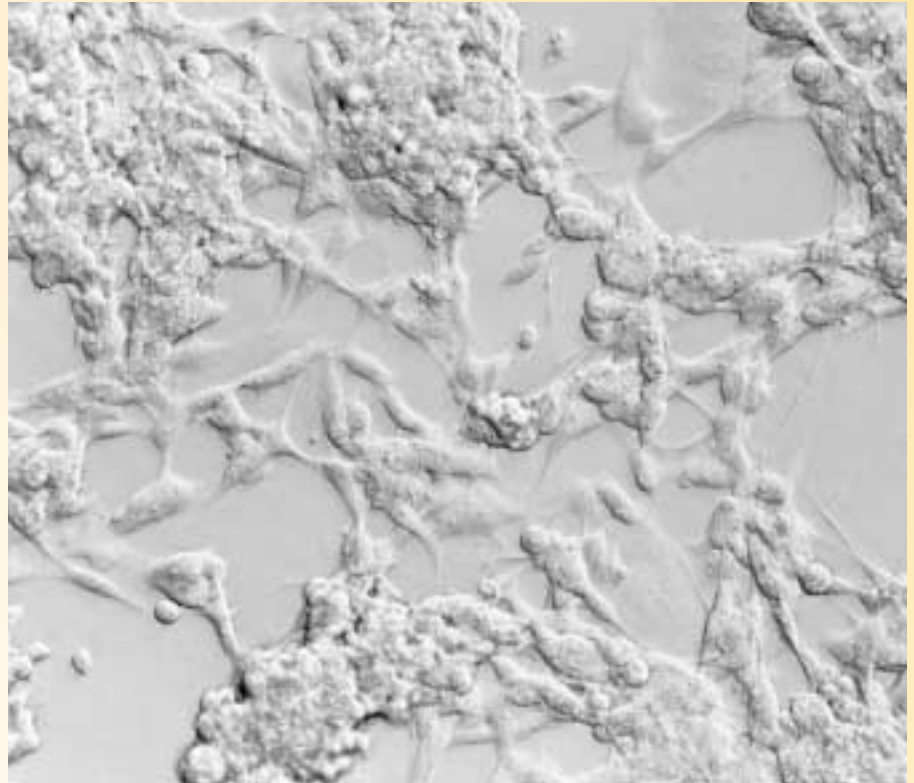
Quality Control:

- ▶ Tested for ability to promote differentiation (neurite outgrowth) of NG-108 rat glioma/mouse neuroblastoma cells
- ▶ Tested and found negative for bacteria and fungi

Storage and Stability:

Stable for at least three months at 2° to 8°C. Do not freeze.

Effects of BD BioCoat EHS Matrix Cellware on NG-108 Rat Glioma/Mouse Neuroblastoma Cells



NG-108 rat glioma/mouse neuroblastoma cells cultured on BD BioCoat EHS Matrix Cellware.

References

1. Early, E.M., et al., Third Intl. Conf. on the Mol. Bio. and Path. of Matrix. Philadelphia, PA (1990).
2. Bowman, et al., Blood. **92(6)**:1941(1998).
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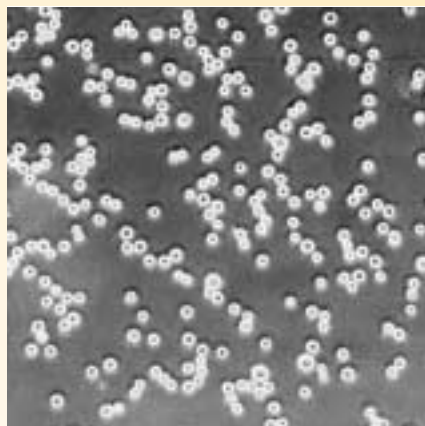
BD BioCoat™ Fibronectin Cellware

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.

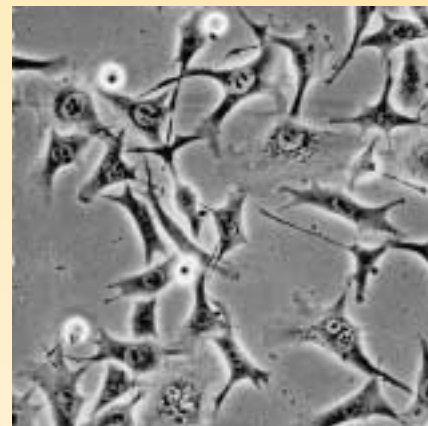
BD BioCoat™ Fibronectin Cellware applications include:

- ▶ 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

Effects of BD BioCoat Fibronectin Cellware on BHK-21 Cells



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



BHK-21 fibroblasts cultured on BD BioCoat Fibronectin CultureSlides attach and spread within one hour.

BD BioCoat™ Fibronectin Cellware 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 and Stability:

Stable for at least three months from date of shipment when stored at 2° to 8°C. Do not freeze.

References

1. Guller, S., et al., *Endocrinology* **130**:2609 (1992).
2. Lavoie, J.N., et al., *J. Cell Biol.* **150**:1037 (2000).
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).
6. Shaw, R.J., *J. Biol. Chem.* **273**:7757 (1998).
7. Yoneda, T., et al., *J. Clin. Invest.* **99**:2509 (1997).

BD BioCoat™ Laminin Cellware

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.

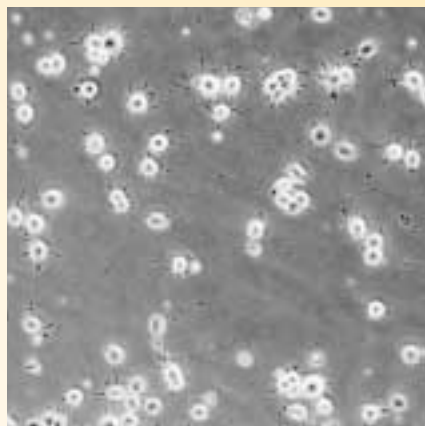
BD BioCoat™ Laminin Cellware applications include:

- ▶ 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

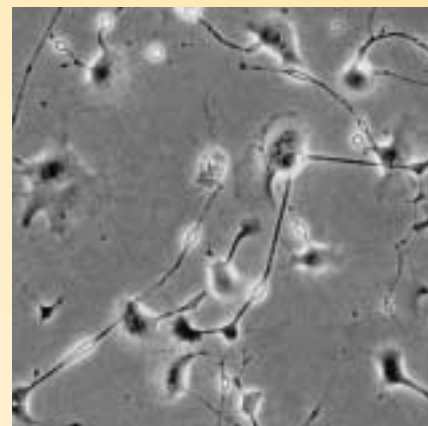
BD BioCoat™ Laminin Cellware has been used to culture:

- ▶ 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

Effects of BD BioCoat Laminin Cellware 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 BD BioCoat Laminin Cellware exhibit a spindle-shaped morphology and dendritic processes.

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 and Stability:

Stable for at least three months from date of shipment when stored at 2° to 8°C. Do not freeze.

References

1. Ocalan, M., et al., Dev. Biol. **125**:158 (1988).
2. Leventhal, P.S. and Feldman, E.L., J. Biol. Chem. **271**:5957 (1996).
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BD BioCoat™ Matrigel™ Matrix Cellware

BD 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. BD 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.

BD BioCoat™ Matrigel™ Matrix Cellware applications include:

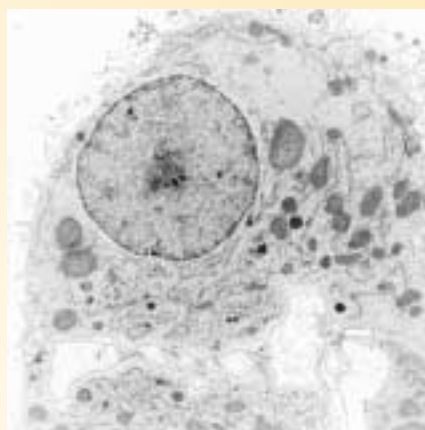
- ▶ Elicitation of tissue-specific cellular morphology and protein production in epithelial cells
- ▶ Differentiation of endothelial, muscle and neuronal cells
- ▶ Development of three-dimensional matrix model systems

BD BioCoat™ Matrigel™ Matrix 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

Rat Hepatocytes Cultured in the BD BioCoat Hepatocyte Differentiation Environment

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



Intracellular structures active nucleus (5000x)

Numerous mitochondria with calcium deposits; rough ER stacks near cell surfaces and surrounding mitochondria; glycogen stores; golgi, ribosomal rosette, lipid droplets.



Intercellular structures (8600x)

Frequent interdigitation of apposing cells; gap and tight junctions; intercellular lumens with microvilli, characteristic of bile canaliculi.

Source:

EHS mouse tumor

Formulation:

Dulbecco's Modified Eagles' Medium with 50 µg/ml gentamycin. BD 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:

- ▶ Cellware stable for at least three months at -20°C. Keep frozen until use.
- ▶ Thin layer cellware stable for at least three months from date of shipment when stored at 2° to 8°C.

For more information on BD Matrigel Matrix, please request literature on our Extracellular Matrices ("Driving Cellular Communications").

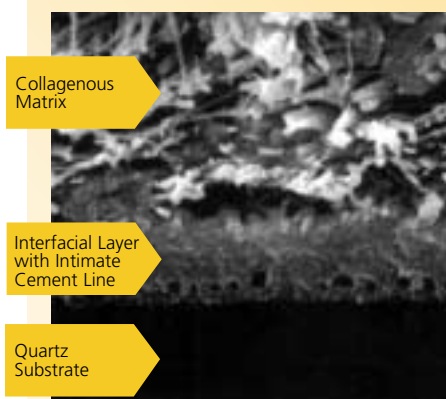
References

1. Fabrega, A.J., et al., Transplantation **62(12)**:1866 (1996).
2. Krams, S.M., et al., Transplantation **65(5)**:713 (1998).
3. Lee, E.J., et al., Neurosurgery **46(6)**:1461 (2000).
4. Reddy, P.M., et al., Pediatric Research **39(2)**:287 (1996).
5. Solomon, L.Z., et al., J. Lab. & Clin. Medicine **132(4)**:279 (1998).
6. Weintraub, A.S., et al., Lab. Invest. **80(11)**:1603 (2000).

BD BioCoat™ Osteologic™ Bone Cell Culture System

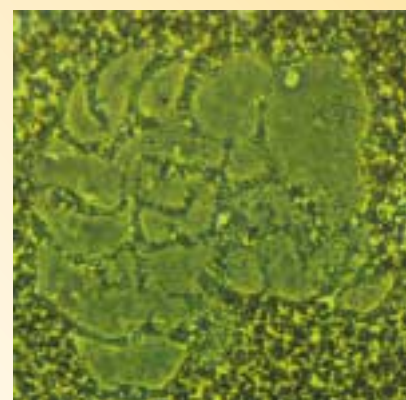
Historically, biologically derived hard tissues such as de-vitalized bone, dentine or ivory slices are used as culture substrates for *in vitro* assays. However, preparation and handling of these matrices is often expensive and time consuming. Ceramic biomaterial substrates are now being considered as an alternative to biologically derived substrates. BD BioCoat™ Osteologic™ Bone Cell Culture System consists of sub-micron synthetic calcium phosphate thin films coated onto various culture vessels. This system has been used as an alternative method for compound screening for direct assessment of osteoclast¹⁻⁴ and osteoblast⁵ activity *in vitro*. The thin film design permits easy and reliable quantification of results.

Osteoclasts - Resorption



Assessment of new bone tissue on BD BioCoat Osteologic Substrate using freeze-fractured cross-section SEM. Note the formation of the cement line.

Osteoclasts - Bone Growth



Osteoclast resorption event in a chamber from a 16-well BD BioCoat™ Osteologic™ slide. This complex resorption pattern closely resembles that seen in normal bone tissue.

BD BioCoat™ Osteologic™ MultiTest Slides

This unique multi-well test format is ideal for screening applications. Also designed for customers requiring multiple assays on a common substrate, and for parallel tests of resorption and bone growth *in vitro*.

- ▶ Proprietary bone biomaterial
- ▶ High test density — 16 discrete wells per quartz slide
- ▶ Allows parallel resorption and bone growth studies — minimizes variables
- ▶ Machine readable results using Microst™ Image Analyzer*
- ▶ Cost effective

BD BioCoat™ Osteologic™ Discs

This unique system incorporates a resorbable artificial bone analog in the form of sub-micron calcium phosphate films on transparent quartz substrates.

- ▶ Proprietary bone biomaterial
- ▶ Direct assessment of osteoclast and osteoblast activity *in vitro*
- ▶ Machine readable results using Microst™ Image Analyzer*
- ▶ Coverslip configurations also available for confocal microscopy, immunofluorescence and electrophysiology studies

* Please contact your local BD Biosciences representative for additional information.

BD BioCoat™ Osteologic™ Disposables Technical Specifications

Film Composition: Proprietary Calcium Phosphates

Film Thickness: Approximately 0.6 µm

Dimensions: Discs — 12.7 mm diam. x 1.0 mm thick

Slides — 76.0 mm x 25.0 x 1.0 mm thick

Coverslips — 12.7 mm diam. x 0.16 mm thick

Quality Control:

- ▶ Tested for resorptive activity by rat primary osteoclasts
- ▶ Sterilized by ETO gas

References:

1. Gu, W., et al., Fifth World Biomaterials Congress (1996).
2. Kurihara, N., et al., Experimental Hematology **26**:1080 (1998).
3. Loomer, P.M., et al., ASBMR 18th Annual Meeting Abstract (1996).
4. Schneider, M.A., et al., ASBMR 18th Annual Meeting Abstract (1996).
5. Sindrey, D., et al., ASBMR 21st Annual Meeting Abstract (1999).

BD BioCoat™ Poly-D-Lysine/Laminin, Poly-L-Ornithine/Laminin and Laminin/Fibronectin Cellware

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.

BD BioCoat™ PDL/LM and PLO/LM Cellware 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. BD BioCoat LM/HFN Cellware provides an *in vitro* environment that promotes cell attachment and extensive process formation.

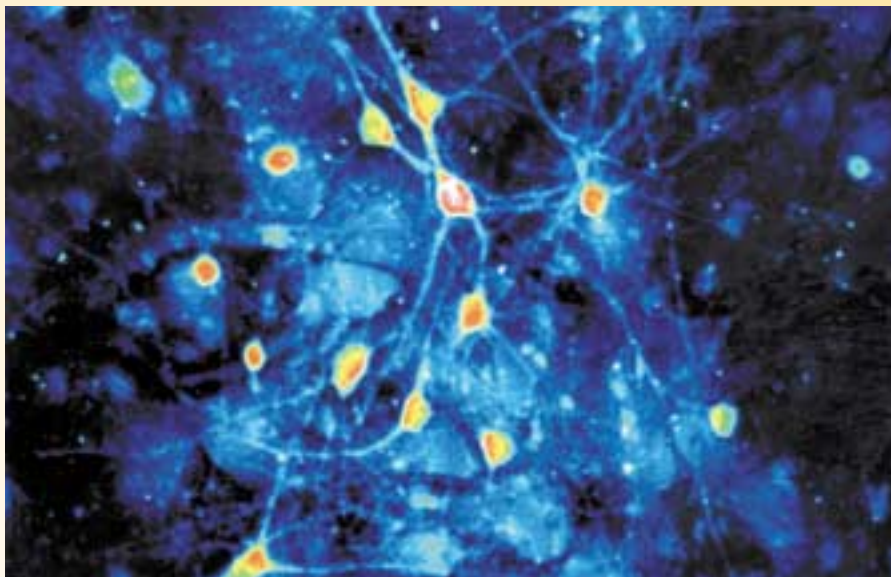
BD BioCoat™ PDL/LM, PLO/LM and LM/HFN Cellware applications include:

- ▶ 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

BD 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 DRGNs^{3,4}
- ▶ Transfected PC12 cells⁴
- ▶ MCF-10A cells⁵
- ▶ Rat primary dorsal root ganglion neurons⁶

Effects of BD BioCoat LM/HFN on Primary Brain Cells



Neurotmitter induced calcium waves in CNS cells plated on BD BioCoat Laminin/Fibronectin.

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

Storage and Stability:

Stable for at least three months at 2° to 8°C. Do not freeze.

Quality Control:

- ▶ PDL/LM and PLO/LM tested for ability to initiate differentiation (neurite outgrowth) of NG-108 rat glioma/mouse neuroblastoma cells
- ▶ Tested and found negative for bacteria and fungi

References

1. Leventhal, P.S. and Feldman, E.L., J. Biol. Chem. **271**:5957 (1996).
2. Maiese, K., et al., J. Neurosci. **13**:3034 (1993).
3. Nakashima, K., et al., J. Neurosci. **19**:5429 (1999).
4. Riederer, B.M., et al., PNAS USA, **94**:741 (1997).
5. Salas, P.J., et al., J. Cell Biol. **137**:359 (1997).
6. Tanner, S.L., et al., J. Neurochem. **75**:553 (2000).

BD 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. BD BioCoat™ T-Cell Activation Plates are precoated with high-quality BD Pharmingen CD3 antibodies. Available for use with mouse or human T-cells, BD BioCoat™ T-Cell Activation Plates offer lot-to-lot consistency and come individually packaged with lids for ease of use.

BD BioCoat™ T-Cell Activation Plate applications include:

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

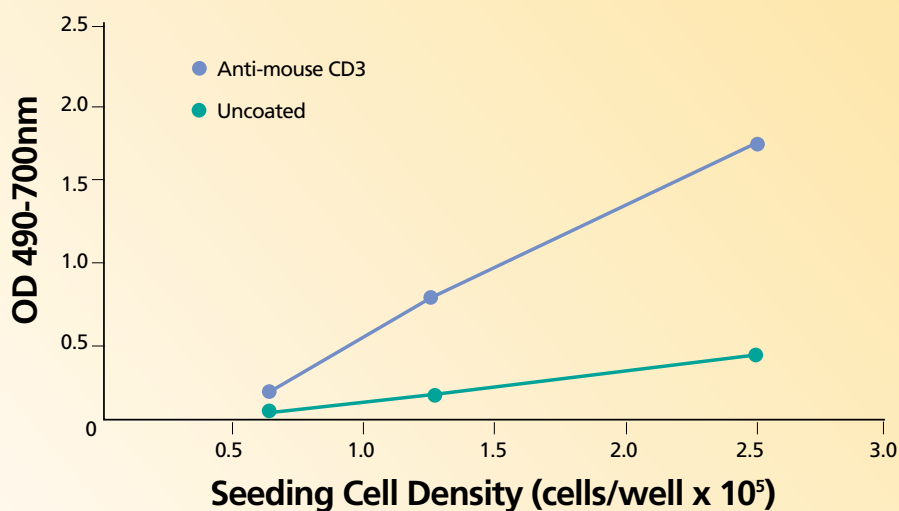
Quality Control:

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

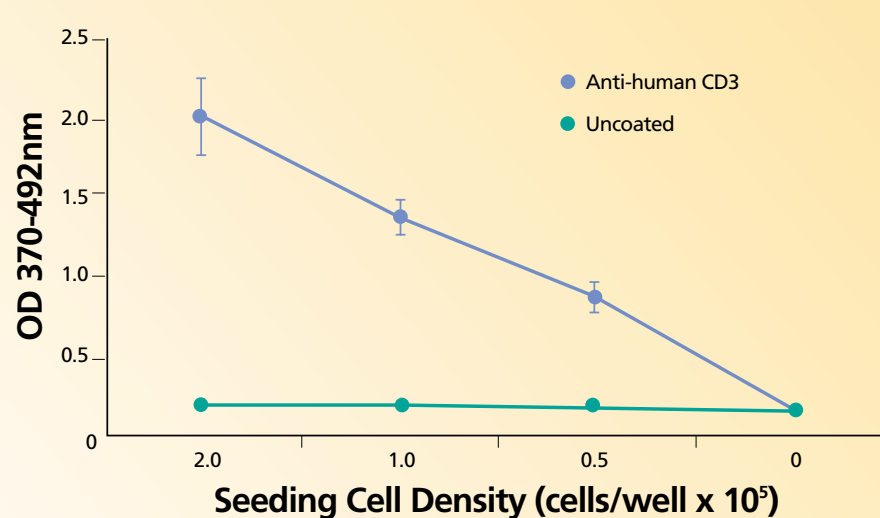
Storage and Stability:

Stable for at least three months at 2° to 8°C. Do not freeze.

Anti-Mouse CD3 Plates



Anti-Human CD3 Plates



BD BioCoat™ Variety Pack Cellware Products/ Vented Caps for BD BioCoat™ Flasks

BD BioCoat™ Variety Packs each contain 6-well Multiwell Plates or CultureSlides with a selection of different extracellular matrix proteins and attachment factors.

BD BioCoat™ Variety Pack Cellware 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 BD BioCoat Cellware product)
- ▶ Tested and found negative for bacteria and fungi

Storage and Stability:

Stable for at least three months at 2° to 8°C. Do not freeze.

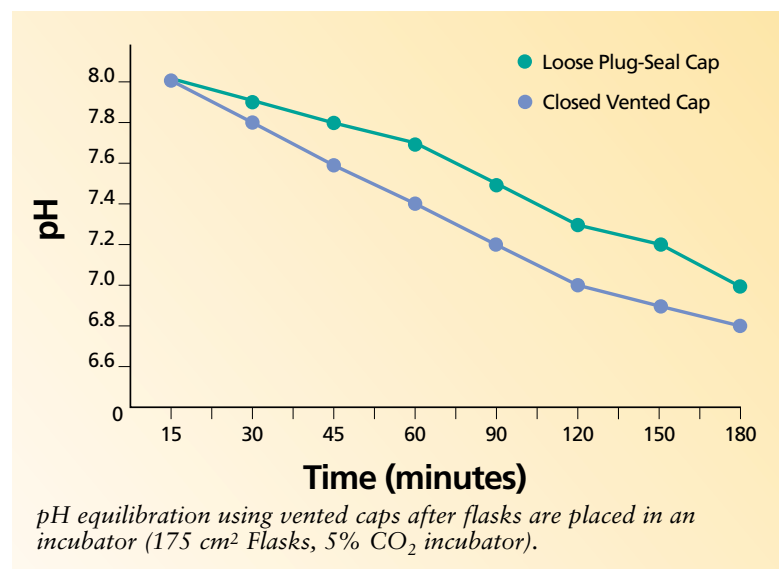


Vented Caps for BD BioCoat™ Flasks

Vented caps are available for use with BD 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 & Stability:

Store at ambient temperature.



BD BioCoat™ Collagen and PDL 96- and 384-well Plates for High Throughput Screening

Application Focus: Analysis of Transfected Cell Lines

BD 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- or 384-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 BD BioCoat PDL [Figure on next page], PLL or Collagen I Cellware. BD BioCoat Cellware 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 BD BioCoat PDL and Collagen I, respectively. Also, a variety of BD BioCoat products are available that support neurite outgrowth and neurotransmitter receptor function.

High throughput studies of a variety of cell types (transfected and untransfected) have also been carried out using BD BioCoat HFN, Collagen IV and PDL/LM. Overall, BD BioCoat Cellware 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

BD BioCoat 96- and 384-well Microplates



BD Biosciences Discovery Labware offers a wide selection of microplates for cell-based fluorescence, luminescence, colorimetric and radiometric assays.

For a complete listing of High Throughput Screening products please visit our web site or see our Drug Discovery Catalog.

Culture Substrates for Transfected Cells

Cell Attachment Substrate	Cell Type
BD BioCoat™ Poly-D-Lysine	HEK-293 293 EBNA Cardiomyocyte Human Astrocytoma (1321N1) Mouse Pituitary (AtT-20) Pancreatic Islet (RIN-m) COS-7
BD BioCoat Poly-L-Lysine	HEK-293 PC12
BD BioCoat Collagen I	HEK-293 PC12
BD BioCoat Fibronectin	Pancreatic Tumor (AR42J) COS-7
BD Cell-Tak™ Cell and Tissue Adhesive	HEK-293 L9 Mouse Fibroblasts

Specialized experimental conditions or the unique properties of a transfected cell line may result in poor adhesion to poly-D-lysine or other cell attachment substrates. In these cases, the BD BioCoat Custom Coating Service is dedicated to meeting your needs by developing specialized formulations of ECM proteins and/or cell attachment molecules.

Improved HEK-293 Cell Adhesion Post-Transfection with BD BioCoat™ Assay Plates



Transfected HEK-293 cells exhibit poor adhesion to tissue culture-treated 96-well assay plates following multiple washes (top row). In contrast, these cells exhibit strong attachment to BD BioCoat PDL 96-well assay plates following vigorous washing (bottom row).

BD BioCoat™ Services



BD BioCoat™ Custom Coating Service

Custom Coating, the way YOU want it. BD BioCoat Custom Coating Service offers an extensive selection of cell-based coatings on a wide variety of BD Falcon™ vessels — from roller bottles to flasks to 384-well plates. We can even develop special coatings for you. Simply tell us the type of vessel and coating you would like applied and we'll make a trial sample for you to evaluate.

BD BioCoat™ Consulting Service

BD Biosciences is committed to helping you optimize your cell culture conditions. The process of selecting the most appropriate substratum



is often empirical and time consuming. Our consulting service can facilitate this process for you.

Our highly trained technical service staff will assist you in determining which BD BioCoat product is best for your cell type and application. Let BD Biosciences put its comprehensive technical database and expertise in cell culture to work for you.

BD BioCoat™ Bar Coding Service

Get on the road to increased productivity with BD BioCoat. The BD Biosciences Discovery



Labware Bar Coding Service provides high-quality bar code labels affixed to any

side of a microplate. Bar codes have been quality tested for optimal readability, chemical resistance and temperature durability.

Ordering Information

Cat. No.	Description	Qty/Pack
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BD BioCoat™ Collagen I Cellware Multiwell and Assay Plates

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
354649	96-well black/clear	5
356649	96-well black/clear	50
354650	96-well white/clear	5
356650	96-well white/clear	50
354519	96-well white	5
356519	96-well white	50
354666	384-well clear	5
356666	384-well clear	50
354667	384-well black/clear	5
356667	384-well black/clear	50
354664	384-well white/clear	5
356664	384-well white/clear	50
354665	384-well white	5
356665	384-well white	50

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

354531	25 cm ² , plug-seal cap†	10
356531	25 cm ² , plug-seal cap†	50
354484	25 cm ² , vented-cap cap†	10
356484	25 cm ² , vented-cap cap†	50
354462	75 cm ² , plug-seal cap†	5
356462	75 cm ² , plug-seal cap†	50
354485	75 cm ² , vented-cap cap†	5
356485	75 cm ² , vented-cap cap†	50
354645	150 cm ² , plug-seal cap	5
356645	150 cm ² , plug-seal cap	40
354486	150 cm ² , vented-cap cap	5
356486	150 cm ² , vented-cap cap	40
354478	175 cm ² , plug-seal cap	5
356478	175 cm ² , plug-seal 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

354556	1-well	12
354627	2-well	12
354557	4-well	12
354630	8-well	12

Cat. No.	Description	Qty/Pack
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BD BioCoat™ Poly-D-Lysine Cellware 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
354640	96-well black/clear	5
356640	96-well black/clear	50
354651	96-well white/clear	5
356651	96-well white/clear	50
354620	96-well white	5
356620	96-well white	50
354662	384-well clear	5
356662	384-well clear	50
354663	384-well black/clear	5
356663	384-well black/clear	50
354660	384-well white/clear	5
356660	384-well white/clear	50
354661	384-well white	5
356661	384-well white	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

354479	25 cm ² , plug-seal cap†	10
356479	25 cm ² , plug-seal cap†	50
354536	25 cm ² , vented-cap†	10
356536	25 cm ² , vented-cap†	50
354524	75 cm ² , plug-seal cap†	5
356524	75 cm ² , plug-seal cap†	50
354537	75 cm ² , vented-cap†	5
356537	75 cm ² , vented-cap†	50
354495	150 cm ² , plug-seal cap	5
356495	150 cm ² , plug-seal cap	40
354538	150 cm ² , vented-cap	5
356538	150 cm ² , vented-cap	40
354529	175 cm ² , plug-seal cap	5
356529	175 cm ² , plug-seal 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

354566	1-well	12
354629	2-well	12
354577	4-well	12
354632	8-well	12

Cat. No.	Description	Qty/Pack
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BD BioCoat™ Poly-L-Lysine Cellware 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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BD BioCoat™ Gelatin Cellware Multiwell and Assay Plates

354652	6-well	5
356652	6-well	50
354689	96-well	5
356689	96-well	50

Culture Dishes

354653	100 mm	10
356653	100 mm	40

Flasks

354654	75 cm ² , plug-seal cap†	5
356654	75 cm ² , plug-seal cap†	50
354488	75 cm ² , vented-cap cap†	5
356488	75 cm ² , vented-cap cap†	50

BD BioCoat™ Collagen IV Cellware 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
354554	150 mm	5

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

BD BioCoat™ EHS Matrix Cellware Multiwell Plates

354418	6-well	5
354419	24-well	5

† BD BioCoat 25 cm² Flasks are 70 ml canted neck; BD BioCoat 75 cm² Flasks are 250 ml canted neck.

Cat. No.	Description	Qty/Pack
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BD BioCoat™ Laminin Cellware 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

BD BioCoat™ Fibronectin Cellware Multiwell and Assay Plates

354402	6-well plates	5
354501	12-well plates	5
354411	24-well plates	5
354506	48-well plates	5
354409	96-well plates	5

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

354558	1-well	12
354628	2-well	12
354559	4-well	12
354631	8-well	12

Cat. No.	Description	Qty/Pack
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BD BioCoat™ Laminin/Fibronectin Cellware Assay Plates

354670	96-well	5
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BD BioCoat™ Matrigel™ Matrix Cellware 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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BD BioCoat™ Matrigel™ Matrix Cellware — 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

BD BioCoat™ Matrigel™ Matrix Cellware for Hepatocytes Multiwell Plates

354510	6-well	5
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Culture Dishes

354634	100 mm	5
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BD BioCoat™ GFR Matrigel™ Matrix Cellware for Smooth Muscle Cells Multiwell Plates

354635	24-well	5
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BD BioCoat™ Osteologic™ Bone Cell Culture System Multitest Slides

354608	16-well	2
354609	16-well	8

Discs

354610	12.7 mm discs in a 24-well plate	24
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Coverslips

354611	12 mm round	5
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Cat. No.	Description	Qty/Pack
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BD BioCoat™ Poly-D-Lysine/Laminin Cellware 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

354687	2-well	12
354688	8-well	12

BD BioCoat™ Poly-L Ornithine/ Laminin Cellware Multiwell and Assay Plates

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

BD BioCoat™ T-Cell Activation 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 BD BioCoat™ Flasks

354637	25 cm ² , vented cap	100
354638	75 cm ² , vented cap	100
354639	175 cm ² , vented cap	50

BD BioCoat™ Variety Pack Cellware Multiwell Plates

354417	6-well <i>includes: Collagen I, Fibronectin, Laminin, Poly-D-Lysine and BD Falcon™ Plates</i>	5
354431	6-well <i>includes: Collagen I, Collagen IV, Fibronectin, Laminin and Poly-D-Lysine Plates</i>	5

CultureSlides

354655	2-well <i>includes: Collagen I, Fibronectin, Poly-D-Lysine and BD Falcon™ CultureSlides</i>	12
354656	8-well <i>includes: Collagen I, Fibronectin, Poly-D-Lysine and BD Falcon™ CultureSlides</i>	12

For BD BioCoat™ Cell Culture Inserts and Assay Systems, please see our general BD Falcon™/BD BioCoat™ catalog.

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