

GUIDELINES FOR USE

PRODUCT: BD Matrigel™ Basement Membrane Matrix Growth Factor Reduced, 10 ml vial
CATALOG NUMBER: 354230

BACKGROUND:

Basement membranes are thin extracellular matrices underlying cells *in vivo*. BD Matrigel Matrix Growth Factor Reduced is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins. Its major component is laminin, followed by collagen IV, heparan sulfate proteoglycans, entactin/nidogen.^{1,2} BD Matrigel Matrix Growth Factor Reduced also contains TGF-beta, epidermal growth factor, insulin-like growth factor, fibroblast growth factor, tissue plasminogen activator,^{3,4} and other growth factors which occur naturally in the EHS tumor. BD Matrigel Matrix Growth Factor Reduced is effective for the attachment and differentiation of both normal and transformed anchorage dependent epithelioid and other cell types. These include neurons,^{5,6} hepatocytes,⁷ Sertoli cells,^{8,9} chick lens,¹⁰ and vascular endothelial cells.¹¹ BD Matrigel Matrix Growth Factor Reduced will influence gene expression in adult rat hepatocytes^{12,13} as well as three dimensional culture in mouse¹⁴⁻¹⁷ and human^{18,19} mammary epithelial cells. It is the basis for several types of tumor cell invasion assays,^{20,21} will support *in vivo* peripheral nerve regeneration,²²⁻²⁴ and provides the substrate necessary for the study of angiogenesis both *in vitro*^{25,26} and *in vivo*.²⁷⁻²⁹ BD Matrigel Matrix Growth Factor Reduced also supports *in vivo* propagation of human tumors in immunosuppressed mice.³⁰⁻³² For further information, go to our website at www.bdbiosciences.com.

BD Matrigel Matrix Growth Factor Reduced was developed for those who require a reconstituted basement membrane preparation purified and characterized to a greater extent than BD Matrigel Matrix. The method³³ used to prepare this product effectively reduced the level of a variety of growth factors except for TGF-beta which may be bound to collagen IV³⁴ and/or sequestered in a latent form that partitions with the major components in the purification procedure. The major components: laminin, collagen IV and entactin (nidogen) are conserved by the process while the level of heparan sulfate proteoglycan is reduced by 40-50%. The following table shows the values for growth factors in BD Matrigel Matrix compared to a typical lot of BD Matrigel Matrix Growth Factor Reduced.

Parameter	BD Matrigel Matrix	BD Matrigel Matrix Growth Factor Reduced
bFGF (pg/ml) ⁴	0 - 0.1	0 - 0.1
EGF (ng/ml)	0.5 - 1.3	< 0.5
IGF-1 (ng/ml)	15.6	5
PDGF (pg/ml)	12	< 5
NGF (ng/ml)	< 0.2	< 0.2
TGF-beta (ng/ml)	2.3	1.7
% Protein that gels	80	83

SOURCE: Engelbreth-Holm-Swarm (EHS) Mouse Tumor
FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/ml gentamycin
 Matrigel Basement Membrane Matrix is compatible with all culture media
STABILITY: Stable for a minimum of three months from day of shipment when stored at -20°C
KEEP FROZEN

**RECONSTITUTION
AND USE:**

Color variations may occur in frozen or thawed vials of BD Matrigel Matrix Growth Factor Reduced, ranging from straw yellow to dark red due to the interaction of carbon dioxide with the bicarbonate buffer and phenol red. Variation in color is normal, does not affect product efficacy, and will disappear upon equilibration with 5% CO₂.

Once BD Matrigel Matrix Growth Factor Reduced is thawed, swirl vial to be sure that material is evenly dispersed. Handle using sterile technique. Place thawed vial of BD Matrigel Matrix Growth Factor Reduced in sterile area, spray top of vial with 70% ETOH and air dry. BD Matrigel Matrix Growth Factor Reduced may be gently pipetted using a pre-cooled pipette to ensure homogeneity.

BD Matrigel Matrix Growth Factor Reduced may be used as a thin gel layer (0.5mm), with cells plated on top. Cells may also be cultured inside the BD Matrigel Matrix Growth Factor Reduced, using a 1 mm layer. Extensive dilution will result in a thin, non-gelled protein layer. This may be useful for cell attachment, but may not be as effective in differentiation studies.

Dispense remaining material into appropriate aliquots, using pre-cooled tubes, and refreeze immediately. Avoid multiple freeze thaws. **DO NOT STORE IN FROST-FREE FREEZER.**

CAUTION:

BD Matrigel Matrix Growth Factor Reduced will gel rapidly at 22°C to 35°C. Thaw overnight at 4°C on ice (Matrigel may gel at slightly elevated temperatures in a refrigerator). Keep product on ice before use, and use pre-cooled pipettes, tips, and tubes when preparing BD Matrigel Matrix Growth Factor Reduced for use. Gelled BD Matrigel Matrix Growth Factor Reduced may be re-liquified if placed at 4°C on ice for 24-48 hours.

COATING PROCEDURES:

BD Matrigel Matrix Growth Factor Reduced may be used in several ways. The Thin Gel Method is useful for plating cells on top of the gel, the Thick Gel Method allows you to grow cells within a three dimensional matrix, and the Thin Coating Method (no gel) provides you with a complex protein layer on top of which to grow your cells. Make your selection based on the final result that you wish to achieve, whether it is cell growth, attachment or differentiation.

NOTE: Some investigators prefer to dilute Matrigel Basement Membrane Matrix. If you wish to maintain a gelled consistency, do not dilute more than 1:3. Use serum-free medium to dilute Matrigel Basement Membrane Matrix. Once gelled, Matrigel Basement Membrane Matrix should be used immediately.

Thin Gel Method

1. Thaw BD Matrigel Matrix Growth Factor Reduced as recommended. Using cooled pipettes, mix the BD Matrigel Matrix Growth Factor Reduced to homogeneity.
2. Keeping culture plates on ice, add 50 µL per square centimeter of growth surface.
3. Place plates at 37°C for 30 minutes. Plates are now ready to use.

Thick Gel Method

1. Thaw BD Matrigel Matrix Growth Factor Reduced as recommended. Using cooled pipettes, mix the BD Matrigel Matrix Growth Factor Reduced to homogeneity.
2. Keep culture plates on ice. Add cells to BD Matrigel Matrix Growth Factor Reduced and suspend using cooled pipettes. Add 150-200 µL per square centimeter of growth surface.
3. Place plates at 37°C for 30 minutes. Culture medium may now be added. Cells may also be cultured on top of this gel.

Thin Coating Method

1. Thaw BD Matrigel Matrix Growth Factor Reduced as recommended. Using cooled pipettes, mix the BD Matrigel Matrix Growth Factor Reduced to homogeneity.
2. Dilute BD Matrigel Matrix Growth Factor Reduced to desired concentration using serum-free medium. Empirical studies should be completed to determine the optimal coating concentration for your application.
3. Add diluted BD Matrigel Matrix Growth Factor Reduced to vessel being coated. Quantity should be sufficient to cover entire growth surface easily. Incubate at room temperature for one hour.
4. Aspirate unbound material and rinse gently using serum-free medium. Plates are now ready to use.

CELL RECOVERY:

Dispase (Catalog No. 354235), BD Cell Recovery Solution (Catalog No. 354253)

Most efficient recovery of cells growing on BD Matrigel Matrix Growth Factor Reduced is accomplished using BD Cell Recovery Solution that depolymerizes the Matrigel Matrix within 7 hours on ice or with Dispase, a metalloenzyme which gently releases the cells allowing for continuous culture.

REFERENCES:

1. Kleinman, H.K., et al., Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma, *Biochemistry*, **21**:6188 (1982).
2. Kleinman, H.K., et al., Basement membrane complexes with biological activity, *Biochemistry*, **25**:312 (1986).
3. Vukicevic, S., et al., Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular activity related to extracellular matrix components, *Experimental Cell Research*, **202**:1 (1992).
4. McGuire, P.G. and Seeds, N.W., The interaction of plasminogen activator with a reconstituted basement membrane matrix and extracellular macromolecules produced by cultured epithelial cells, *J. Cell. Biochem.*, **40**:215 (1989).
5. Biederer, T. and Scheiffele, P., Mixed-culture assays for analyzing neuronal synapse formation, *Nature Protocols*, **2**(3):670 (2007).
6. Li, Y., et al., Essential Role of TRPC channels in the guidance of nerve growth cones by brain-derived neurotrophic factor, *Nature*, **434**:894 (2005).
7. Bi, Y., et al., Use of cryopreserved human hepatocytes in sandwich culture to measure hepatobiliary transport, *Drug Metab. and Dispos.*, **34**(9):1658 (2006).
8. Hadley, M.A., et al., Extracellular matrix regulates sertoli cell differentiation, testicular cord formation, and germ cell development in vitro, *J. Cell Biol.*, **101**:1511 (1985).
9. Yu, X., et al., Essential role of extracellular matrix (ECM) overlay in establishing the functional integrity of primary neonatal rat sertoli cell/gonocyte co-cultures: An improved in vitro model for assessment of male reproductive toxicity, *Toxicological Sciences*, **84**(2):378 (2005).
10. Ireland, M.E., Quantification and regulation of mRNAs encoding beaded filament proteins in the chick lens, **16**(8):838 (1997).
11. McGuire, P.G., and Orkin, R.W., A simple procedure to culture and passage endothelial cells from large vessels of small animals, *Biotechniques*, **5**(6):456 (1987).
12. Bissel, D.M., et al., Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver, *J. Clinical Invest.*, **79**:801 (1987).
13. Page, J.L., et al., Gene expression profiling of extracellular matrix as an effector of human hepatocyte phenotype in primary cell culture, *Toxicological Sciences*, **97**(2):384 (2007).
14. Li, M.L., et al., Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells, *Proc. Nat. Acad. Sci. USA*, **84**:136 (1987).
15. Barcellof, M.H., et al., Functional differentiation and aveolar morphogenesis of primary mammary cultures on reconstituted basement membrane, *Development*, **105**:223 (1989).
16. Roskelley, C.D., et al., Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction, *Proc. Nat. Acad. Sci. USA*, **91**(26):12378 (1994).
17. Xu, R., et al., Extracellular matrix-regulated gene expression requires cooperation of SWI/SNF and transcription factors, *J. Biol. Chem.*, **282**(20):14992 (2007).
18. Debnath, J., et al., Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures, *Methods*, **30**(3):256 (2003).
19. Muthuswamy, S.K., et al., ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini, *Nat. Cell Biol.*, **3**(9):785 (2001).
20. Terranova, V.P., et al., Use of a reconstituted basement membrane to measure cell invasiveness and select for highly invasive tumor cells, *Proc. Nat. Acad. Sci. USA*, **83**:465 (1986).
21. Albin, A., et al., A rapid in vitro assay for quantitating the invasive potential of tumor cells, *Cancer Research*, **47**:3239 (1987).
22. Madison, R., et al., Increased rate of peripheral nerve regeneration using bioresorbable nerve guides and laminin containing gel, *Exp. Neurology*, **88**:767 (1985).
23. Xu, X.M., et al., Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord, *J. Comp. Neurol.*, **351**(1):145 (1994).
24. Fouad, K., et al., Combining schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord, *The Journal of Neuroscience*, **25**(5):1169 (2005).

25. Kubota, Y., et al., Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures, *J. Cell Biol.*, **107**:1589 (1988).
26. Maeshima, Y., et al., Identification of the anti-angiogenic site within vascular basement membrane-derived Tumstatin, *J. Biol. Chem.*, **276**(18):15240 (2001).
27. Passaniti, A., et al., A simple, quantitative method for assessing angiogenesis and anti-angiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor, *Lab Invest.*, **67**:519 (1992).
28. Isaji, M., et al., Tranilast inhibits the proliferation, chemotaxis and tube formation of human microvascular endothelial cells in vitro and angiogenesis in vivo, *British Journal of Pharmacology*, **122**:1061 (1997).
29. Kisucka, J., et al., Platelets and platelet adhesion support angiogenesis while preventing excessive hemorrhage, *Proc. Nat. Acad. Sci. USA*, **103**(4):855 (2006).
30. Albini, A., et al., Matrigel promotes retinoblastoma cell growth in vitro and in vivo, *Int. J. Cancer*, **52**(2):234 (1992).
31. Yue, W., et al., MCF-7 human breast carcinomas in nude mice as a model for evaluating aromatase inhibitors, *J. Steroid Biochem. Molec. Biol.*, **44**(4-6):671 (1993).
32. Angelucci, A., et al., Suppression of EGF-R signaling reduces the incidence of prostate cancer metastasis in nude mice, *Endocrine-Related Cancer*, **13**(1):197 (2006).
33. Taub, M., et al., Epidermal growth factor or transforming growth factor α is required for kidney tubulogenesis in matrigel cultures in serum-free medium, *Proc. Nat. Acad. Sci. USA*, **87**: 4002 (1990).
34. Paralkar, V.M., et al., Transforming growth factor beta type 1 binds to collagen IV of basement membrane matrix: implications for development, *Dev. Biol.*, **143**: 303 (1991).

CALIFORNIA PROPOSITION 65 NOTICE

WARNING: This product contains a chemical known to the state of California to cause cancer.

Component: Chloroform

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

BD Biosciences – Discovery Labware

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