

GUIDELINES FOR USE

PRODUCT: Corning® Matrigel® Basement Membrane Matrix Growth Factor Reduced Phenol Red Free, 10 ml vial

CATALOG NUMBER: 356231

BACKGROUND:

Basement membranes are thin extracellular matrices underlying cells *in vivo*. Corning® Matrigel® Matrix Growth Factor Reduced (GFR) Phenol Red Free 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} Corning® Matrigel® Matrix GFR Phenol Red Free 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. Corning® Matrigel® Matrix GFR Phenol Red Free 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 Corning® Matrigel® Matrix GFR Phenol Red Free will influence gene expression in adult rat hepatocytes,^{12,13} vascular endothelial cells,¹⁴ as well as three dimensional culture in mouse¹⁵⁻¹⁸ and human^{19,20} mammary epithelial cells. It is the basis for several types of tumor cell invasion assays,^{21,22} will support *in vivo* peripheral nerve regeneration,²³⁻²⁵ and provides the substrate necessary for the study of angiogenesis both *in vitro*^{26,27} and *in vivo*.^{25, 28-30} Corning® Matrigel® Matrix GFR Phenol Red Free also supports *in vivo* propagation of human tumors in immunosuppressed mice.³¹⁻³³ Corning® Matrigel® Matrix GFR Phenol Red Free can be used for the transplantation of unsorted mammary cells,³⁴ as well as sorted epithelial subpopulations embedded in Corning® Matrigel®.^{35,36} This matrix has also been used as a cancer stem cell model and shown to enhance tumor growth rates *in vivo*.³⁷

Corning® Matrigel® Matrix GFR Phenol Red Free was developed for those who require a reconstituted basement membrane preparation purified and characterized to a greater extent than Corning® Matrigel® Matrix. The method³⁸ 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 IV³⁹ 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 Corning® Matrigel® Matrix compared to a typical lot of Corning® Matrigel® Matrix GFR Phenol Red Free.

Parameter	Corning® Matrigel® Matrix	Corning® Matrigel® Matrix GFR Phenol Red Free
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

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SOURCE: Engelbreth-Holm-Swarm (EHS) Mouse Tumor

FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/mL gentamycin.
Corning® Matrigel® Matrix GFR Phenol Red Free is compatible with all culture media.

STORAGE: Stable when stored at -20°C. Freeze thaws should be minimized by aliquotting into one time use aliquots. Store aliquots in the -20°C freezer until ready for use. **DO NOT STORE IN FROST-FREE FREEZER. KEEP FROZEN.**

EXPIRATION DATE: The expiration date for Corning® Matrigel® Matrix GFR Phenol Red Free is lot specific and can be found on the product Certificate of Analysis.

CAUTION: It is extremely important that Corning® Matrigel® Matrix GFR Phenol Red Free and all cultureware or media coming in contact with Corning® Matrigel® Matrix GFR Phenol Red Free should be pre-chilled/ice-cold since Corning® Matrigel® Matrix GFR Phenol Red Free will start to gel above 10°C. Keep Corning® Matrigel® Matrix on ice at all times.

RECONSTITUTION AND USE:

Thaw Corning® Matrigel® Matrix GFR Phenol Red Free by submerging the vial in ice in a 4°C refrigerator, in the back, overnight. Once Corning® Matrigel® Matrix GFR Phenol Red Free is thawed, swirl vial to ensure that material is evenly dispersed. Keep Corning® Matrigel® Matrix on ice at all times. Handle with sterile technique. Place thawed vial of Corning® Matrigel® Matrix GFR Phenol Red Free in sterile area, spray top of vial with 70% ethanol and air dry.

Corning® Matrigel® Matrix GFR Phenol Red Free may be gently pipetted using a pre-cooled pipet to ensure homogeneity. Aliquot Corning® Matrigel® Matrix GFR Phenol Red Free to tubes, switching tips whenever Corning® Matrigel® Matrix GFR Phenol Red Free is clogging the tip and/or causing the pipet to measure inaccurately. Gelled Corning® Matrigel® Matrix GFR Phenol Red Free may be re-liquified if placed at 4°C in ice for 24-48 hours.

Corning® Matrigel® Matrix GFR Phenol Red Free may be used as a thin gel layer (0.5 mm), with cells plated on top. Cells may also be cultured inside the Corning® Matrigel® Matrix GFR Phenol Red Free, 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.

COATING PROCEDURES:

Corning® Matrigel® Matrix GFR Phenol Red Free 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: Application specific protocols are posted on the support web page.* The protein concentration for Corning® Matrigel® Matrix products is lot specific and provided on the Certificate of Analysis. For consistent results dilute

Corning® Matrigel® Matrix products by calculating the specific protein concentration (mg/mL) required. To maintain a gelled consistency we recommend not diluting Corning® Matrigel® Matrix to less than 3 mg/mL. Use ice-cold serum-free medium to dilute Corning® Matrigel® Matrix. Mix by pipetting up and down or by swirling the vial in ice.

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Thin Gel Method

1. Thaw Corning® Matrigel® Matrix GFR Phenol Red Free as recommended. Using cooled pipets, mix the Corning® Matrigel® Matrix GFR Phenol Red Free to homogeneity.
2. Keeping culture plates on ice, add 50 µl/cm² of growth surface.
3. Place plates at 37°C for 30 minutes.
4. If necessary aspirate unbound material just before use and rinse gently using serum-free medium. Ensure that the tip of the pipet does not scratch the coated surface. Plates are now ready to use.

Thick Gel Method

1. Thaw Corning® Matrigel® Matrix GFR Phenol Red Free as recommended. Using cooled pipets, mix the Corning® Matrigel® Matrix GFR Phenol Red Free to homogeneity.
2. Keep culture plates on ice. Add cells to Corning® Matrigel® Matrix GFR Phenol Red Free and suspend using cooled pipets. Add 150-200 µl/cm² 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 Corning® Matrigel® Matrix GFR Phenol Red Free as recommended. Using cooled pipets, mix the Corning® Matrigel® Matrix GFR Phenol Red Free to homogeneity.
2. Dilute Corning® Matrigel® Matrix GFR Phenol Red Free to desired concentration using serum-free medium. Empirical studies should be completed to determine the optimal coating concentration for your application.
3. Add diluted Corning® Matrigel® Matrix GFR Phenol Red Free 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:

Corning Dispase (Cat. No. 354235), Corning Cell Recovery Solution (Cat. No. 354253).

Most efficient recovery of cells growing on Corning® Matrigel® Matrix GFR Phenol Red Free is accomplished using Corning Cell Recovery Solution that depolymerizes the Corning® Matrigel® Matrix GFR Phenol Red Free within 7 hours on ice or with

Corning Dispase, a metalloenzyme which gently releases the cells allowing for continuous culture.

***NOTE:** For technical resources contact Technical Support at:
tel: 800.492.1110; email: CLSTechServ@corning.com

REFERENCES:

1. Kleinman HK, et al, Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma, *Biochemistry* **21**:6188 (1982).
2. Kleinman HK, 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, *Exp Cell Res* **202**:1 (1992).
4. McGuire PG and Seeds NW, 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, *Nat Protoc* **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 Dispos.* **34**(9):1658 (2006).
8. Gassei K, et al, Immature rat seminiferous tubules reconstructed *in vitro* express markers of Sertoli cell maturation after xenografting into nude mouse hosts, *Mol Hum Reprod.* **16**(2):97 (2010).
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, *Toxicol Sci* **84**(2):378 (2005).
10. Chandrasekher G, and Sailaja D, Differential activation of phosphatidylinositol 3-kinase signaling during proliferation and differentiation of lens epithelial cells, *Invest Ophthalmol Vis Sci.* **44**(10):4400 (2003).
11. McGuire PG, and Orkin RW, A simple procedure to culture and passage endothelial cells from large vessels of small animals, *Biotechniques* **5**(6):456 (1987).

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12. Bissel DM, et al, Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver, *J. Clin Invest.* **79**:801 (1987).
13. Page JL, et al, Gene expression profiling of extracellular matrix as an effector of human hepatocyte phenotype in primary cell culture, *Toxicol Sci* **97**(2):384 (2007).
14. Cooley LS, et al, Reversible transdifferentiation of blood vascular endothelial cells to a lymphatic-like phenotype *in vitro*, *J Cell Sci.* **123**(Pt 21):3808 (2010).
15. Li ML, 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).
16. Barcellof MH, et al, Functional differentiation and aveolar morphogenesis of primary mammary cultures on reconstituted basement membrane, *Development* **105**:223 (1989).
17. Roskelley CD, 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).
18. Xu R, et al, Extracellular matrix-regulated gene expression requires cooperation of SWI/SNF and transcription factors, *J. Biol. Chem.* **282**(20):14992 (2007).
19. 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).
20. Muthuswamy SK, et al, ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini, *Nat. Cell Biol.* **3**(9):785 (2001).
21. Albini A, et al, A rapid *in vitro* assay for quantitating the invasive potential of tumor cells, *Cancer Res.* **47**:3239 (1987).
22. Poincloux R, et al, Contractility of the cell rear drives invasion of breast tumor cells in 3D Matrigel, *Proc Natl Acad Sci USA.* **108**(5):1943 (2011).
23. Madison R, et al, Increased rate of peripheral nerve regeneration using bioresorbable nerve guides and laminin containing gel, *Exp. Neurology* **88**:767 (1985).
24. Xu XM, et al, Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord, *J. Comp. Neurol.* **351**(1):145 (1994).
25. Lopatina T, et al, Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth de novo, *PLoS One* **6**(3):e17899 (2011).
26. 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).
27. Ponce ML, Tube formation: an *in vitro* matrigel angiogenesis assay, *Methods Mol Biol.* **467**:183 (2009).
28. 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).
29. Isaji M, et al, Tranilast inhibits the proliferation, chemotaxis and tube formation of human microvascular endothelial cells *in vitro* and angiogenesis *in vivo*, *Br J Pharmacol* **122**:1061 (1997).
30. Adini A, et al, Matrigel cytometry: a novel method for quantifying angiogenesis *in vivo*, *J Immunol Method.* **342**(1-2):78 (2009).
31. Albini A, et al, Matrigel promotes retinoblastoma cell growth *in vitro* and *in vivo*, *Int. J. Cancer* **52**(2):234 (1992).
32. Yue W, and Brodie A, MCF-7 human breast carcinomas in nude mice as a model for evaluating aromatase inhibitors, *J. Steroid Biochem. Mol. Biol.* **44**(4-6):671 (1993).
33. Angelucci A, et al, Suppression of EGF-R signaling reduces the incidence of prostate cancer metastasis in nude mice, *Endocr-Relat Cancer* **13**(1):197 (2006).
34. Moraes RC, et al, Constitutive activation of smoothened (SMO) in mammary glands of transgenic mice leads to increased proliferation, altered differentiation and ductal dysplasia, *Development* **134**:1231 (2007).
35. Zeng YA, and Nusse R, Wnt proteins are self-renewal factors for mammary stem cells and promote their long-term expansion in culture, *Cell Stem Cell* **6**:568 (2010).
36. Jeselsohn R, et al, Cyclin D1 kinase activity is required for the self-renewal of mammary stem and progenitor cells that are targets of MMTV-ErbB2 tumorigenesis, *Cancer Cell* **17**:65 (2010).
37. Quintana E, et al, Efficient tumor formation by single human melanoma cells, *Nature* **456**:593 (2008).
38. 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).
39. Paralkar, VM, et al, Transforming growth factor beta type 1 binds to collagen IV of basement membrane matrix: implications for development, *Dev. Biol.*, **143**: 303 (1991).

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

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