

CERTIFICATE OF ANALYSIS

PRODUCT: Corning® Matrigel® Basement Membrane Matrix, 5 ml vial

CATALOG NUMBER: 356234

LOT NUMBER: 6095006

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

FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/ml gentamycin

Corning Matrigel Basement Membrane Matrix is compatible with all culture media

STORAGE: Store at -20°C. Avoid multiple freeze-thaws. Do not store in frost-free freezer. **KEEP FROZEN.**

QUALITY CONTROL:

Specification	Criteria	Result
Protein Concentration	Results obtained by Lowry method and represented in mg/ml.	9.3
Endotoxin	Endotoxin units (EU)/ml are measured by Limulus Amoebocyte Lysate assay.	< 1.5
Gelling	Tested for ability to gel quickly and maintain this form with culture medium for a period of 14 days at 37°C.	PASS
Biological Activity	Biological activity is determined using a neurite outgrowth assay. Chick dorsal root ganglia are plated on a 1.0 mm layer of Corning Matrigel Matrix. Tested for a positive neurite outgrowth response after 48 hours without addition of nerve growth factor.	PASS
Sterility	Tested for the presence of bacteria, fungi and mycoplasma.	NEGATIVE
MAP Test	Mouse colonies screened for Sendai, MHV, PVM, TEMV/GDVII, Ectro, Polyoma, MRV/EDIM, LCM, MCMV, M.Ad, Reo, MPV, LDEV/LDHV, MTV, Hantaan, K, RCMV, CARB	NEGATIVE
PCR Test	Tumor source tested for <i>Mycoplasma spp.</i> , <i>Helicobacter</i> , LDEV/LDHV, Sendai, MHV, PVM, MMV/MVM, MPV, Reo (1, 2, 3), MRV/EDIM, Ectro, LCM, K, MTV, Polyoma, Hantaan, Seoul, M. Ad (1, 2), MCMV, Norovirus, TMEV/GDVII, KRV, Toolan's H-1, RCV/SDA Finished goods tested for LDEV/LDHV.	NEGATIVE

Expiration Date: July 11, 2018

SAFETY RECOMMENDATION: Handle in accordance with good industrial hygiene and laboratory safety practices

Quality Assurance

Date

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)
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GUIDELINES FOR USE

PRODUCT: Corning® Matrigel® Basement Membrane Matrix, 5 mL vial

CATALOG NUMBER: 356234

BACKGROUND: Basement membranes are thin extracellular matrices underlying cells *in vivo*. Corning Matrigel Basement Membrane Matrix 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 Basement Membrane Matrix 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 Basement Membrane Matrix 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 Basement Membrane Matrix 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 Basement Membrane Matrix also supports *in vivo* propagation of human tumors in immunosuppressed mice.³¹⁻³³ Corning Matrigel Basement Membrane Matrix 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

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

FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/mL gentamycin.
Corning Matrigel Basement Membrane Matrix 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 Basement Membrane Matrix is lot specific and can be found on the product Certificate of Analysis.

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

RECONSTITUTION AND USE: Color variations may occur in frozen or thawed vials of Corning Matrigel Basement Membrane Matrix, 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.

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Thaw Corning® Matrigel® Basement Membrane Matrix by submerging the vial in ice in a 4°C refrigerator, in the back, overnight. Once Corning Matrigel Basement Membrane Matrix 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 Basement Membrane Matrix in sterile area, spray top of vial with 70% ethanol and air dry.

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

Corning Matrigel Basement Membrane Matrix 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 Basement Membrane Matrix, 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 Basement Membrane Matrix 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.

Thin Gel Method

1. Thaw Corning Matrigel Basement Membrane Matrix as recommended. Using cooled pipets, mix the Corning Matrigel Basement Membrane Matrix to homogeneity.
2. Keeping culture plates on ice, add 50 $\mu\text{l}/\text{cm}^2$ 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 Basement Membrane Matrix as recommended. Using cooled pipets, mix the Corning Matrigel Basement Membrane Matrix to homogeneity.
2. Keep culture plates on ice. Add cells to Corning Matrigel Basement Membrane Matrix and suspend using cooled pipets. Add 150-200 $\mu\text{l}/\text{cm}^2$ 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 Basement Membrane Matrix as recommended. Using cooled pipets, mix the Corning Matrigel Basement Membrane Matrix to homogeneity.

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2. Dilute Corning® Matrigel® Basement Membrane Matrix 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 Basement Membrane Matrix 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 Disperse (Cat. No. 354235), Corning Cell Recovery Solution (Cat. No. 354253).

Most efficient recovery of cells growing on Corning Matrigel Basement Membrane Matrix is accomplished using Corning Cell Recovery Solution that depolymerizes the Corning Matrigel Basement Membrane Matrix within 7 hours on ice or with Corning Disperse, 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

SAFETY RECOMMENDATION: Handle in accordance with good industrial hygiene and laboratory safety practices

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CALIFORNIA PROPOSITION 65 NOTICE

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

Component: **Chloroform**

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