

CERTIFICATE OF ANALYSIS

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

CATALOG NUMBER: 356230

LOT NUMBER: 6319010

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

FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/ml gentamycin
Growth Factor Reduced Corning Matrigel 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. | 8.7 |
| 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 |

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

Expiration Date: January 9, 2019

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 Growth Factor Reduced, 5 mL vial

CATALOG NUMBER: 356230

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

| Parameter | Corning Matrigel Matrix | Corning Matrigel Matrix GFR |
|---------------------------|-------------------------|-----------------------------|
| 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 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 is lot specific and can be found on the product Certificate of Analysis.

CAUTION: It is extremely important that Corning Matrigel Matrix GFR and all cultureware or media coming in contact with Corning Matrigel Matrix GFR should be pre-chilled/ice-cold since Corning Matrigel Matrix GFR 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 Matrix GFR, 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.

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

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

Corning Matrigel Matrix GFR 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, 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 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 as recommended. Using cooled pipets, mix the Corning Matrigel Matrix GFR 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 as recommended. Using cooled pipets, mix the Corning Matrigel Matrix GFR to homogeneity.
2. Keep culture plates on ice. Add cells to Corning Matrigel Matrix GFR 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 as recommended. Using cooled pipets, mix the Corning Matrigel Matrix GFR to homogeneity.
2. Dilute Corning Matrigel Matrix GFR 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 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 is accomplished using Corning Cell Recovery Solution that depolymerizes the Corning Matrigel Matrix GFR 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

Safety Recommendations:

Safety assessment indicates this product is hazardous; refer to SDS [Safety Data Sheet] for safety information. Handle in accordance with good industrial hygiene and laboratory safety practices.

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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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