CERTIFICATE OF ANALYSIS

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

CATALOG NUMBER: 354230

LOT NUMBER: 4216008

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.	**8.0**
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 *Mycoplasma spp.*, *Helicobacter*, 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: September 28, 2016

Quality Assurance _____

Date 09/03/14

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GUIDELINES FOR USE

PRODUCT: Corning® Matrigel® Basement Membrane Matrix Growth Factor Reduced, 10 mL vial

CATALOG NUMBER: 354230

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 mouse[15-18] 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 immunosupressed 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 method[38] 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 IV[39] 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% CO_2.

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 Corning 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/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® 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/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® 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 please email CLSTechServ@Corning.com

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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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