

CERTIFICATE OF ANALYSIS

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

CATALOG NUMBER: 354263

LOT NUMBER: 6291009

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

FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/ml gentamycin
Corning Matrigel Matrix High Concentration 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.	20.47
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: December 11, 2018

Quality Assurance

Date

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GUIDELINES FOR USE

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

CATALOG NUMBER: 354263

BACKGROUND:

Basement membranes are thin extracellular matrices underlying cells *in vivo*. Corning Matrigel Matrix High Concentration 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 High Concentration 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 High Concentration 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 High Concentration 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 High Concentration GFR also supports *in vivo* propagation of human tumors in immunosuppressed mice.³¹⁻³³ Corning Matrigel Matrix High Concentration GFR can be used for the transplantation of unsorted mammary cells,³⁴ as well as sorted epithelial subpopulations embedded in Corning Matrigel Matrix.^{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 High Concentration 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 High Concentration GFR.

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Parameter	Corning Matrigel Matrix	Corning Matrigel Matrix High Concentration 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

- SOURCE:** Engelbreth-Holm-Swarm (EHS) Mouse Tumor
- FORMULATION:** Dulbecco's Modified Eagle's Medium with 50 µg/mL gentamycin. Corning® Matrigel® Matrix High Concentration 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 High Concentration GFR is lot specific and can be found on the product Certificate of Analysis.
- CAUTION:** It is extremely important that Corning Matrigel Matrix High Concentration GFR and all cultureware or media coming in contact with Corning Matrigel Matrix High Concentration GFR is pre-chilled/ice-cold since Corning Matrigel Matrix High Concentration 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 High Concentration 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 High Concentration GFR by submerging the vial in ice in a 4°C refrigerator, in the back, overnight. Once Corning Matrigel Matrix High Concentration 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 High Concentration GFR in sterile area, spray top of vial with 70% ethanol and air dry.
- Corning Matrigel Matrix High Concentration GFR may be gently pipetted using a pre-cooled pipet to ensure homogeneity. Aliquot Corning Matrigel Matrix High Concentration GFR to tubes, switching tips whenever Corning Matrigel Matrix High Concentration GFR is

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clogging the tip and/or causing the pipet to measure inaccurately. Gelled Corning® Matrigel® Matrix High Concentration GFR may be re-liquified if placed at 4°C in ice for 24-48 hours.

Corning Matrigel Matrix High Concentration 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 High Concentration 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. Corning Matrigel Matrix High Concentration GFR can be used to assess *in vivo* angiogenic activity of different compounds by subcutaneous injection into mice (Corning Matrigel Plug Assay).^{2,8,26} The high protein concentration augments the growth of tumors³⁷⁻³⁹ and also allows the Corning Matrigel Plug to maintain its integrity after injection. This keeps the injected tumor and/or angiogenic compounds localized for *in situ* analysis and/or future excision.

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. Ice-cold medium can be added directly to the frozen vial of Corning Matrigel Matrix High Concentration GFR and thawed as recommended in the 'reconstitution and use' section. Mix by pipetting up and down or by swirling the vial in ice.

INJECTION PROTOCOL:

1. It is critical to keep the Corning Matrigel Matrix High Concentration GFR and the Corning Matrigel Matrix/Cell suspension as cold as possible, without freezing, prior to injecting into the mice. It is very important to keep the Corning Matrigel and the Corning Matrigel Matrix/Cell suspension as aseptic as possible throughout the procedure.
2. For each recipient mouse, mix cells (2×10^5 or greater) and Corning Matrigel Matrix High Concentration GFR together in a final volume of 0.5 mL on ice.
3. The cells should be in as small a volume as possible. Typically, 250 μ l ice cold medium containing 2×10^6 cells/mL is mixed with 250 μ l ice cold Corning Matrigel Matrix High Concentration GFR.
4. Inject the cells subcutaneously in athymic mice using a 19G needle for tissue samples and a 23G needle for cultured cells. The injections should be done quickly to prevent the Corning Matrigel Matrix High Concentration GFR from solidifying.
5. Rotate the syringe when withdrawing to prevent leakage. The needles will need to be changed frequently due to blockage.

NOTE: For more details on this application go to www.corning.com/lifesciences to access Document No. CLS-DL-CC-036. (Technical Bulletin 455: Methods for Implantation of Corning Matrigel Matrix into Mice and Tissue Fixation.)

CELL RECOVERY:

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

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Most efficient recovery of cells growing on Corning® Matrigel® Matrix High Concentration GFR is accomplished using Corning Cell Recovery Solution that depolymerizes the Corning Matrigel Matrix High Concentration 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 visit support page at www.corning.com/lifesciences

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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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