

GUIDELINES FOR USE

PRODUCT: BD 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*. BD 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} BD 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. BD 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 BD Matrigel Matrix GFR Phenol Red Free will influence gene expression in adult rat hepatocytes^{12,13} as well as three dimensional culture in mouse¹⁴⁻¹⁷ and human^{18,19} mammary epithelial cells. It is the basis for several types of tumor cell invasion assays,^{20,21} will support *in vivo* peripheral nerve regeneration,²²⁻²⁴ and provides the substrate necessary for the study of angiogenesis both *in vitro*^{25,26} and *in vivo*.²⁷⁻²⁹ BD Matrigel Matrix GFR Phenol Red Free also supports *in vivo* propagation of human tumors in immunosuppressed mice.³⁰⁻³² For further information, go to our website at www.bdbiosciences.com.

BD 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 BD Matrigel Matrix. The method³³ 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 IV³⁴ 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 BD Matrigel Matrix compared to a typical lot of BD Matrigel Matrix GFR Phenol Red Free.

Parameter	BD Matrigel Matrix	BD 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

SOURCE: Engelbreth-Holm-Swarm (EHS) Mouse Tumor
FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/ml gentamycin
 Matrigel Basement Membrane Matrix is compatible with all culture media
STABILITY: Stable for a minimum of three months from day of shipment when stored at -20°C
KEEP FROZEN



**RECONSTITUTION
AND USE:**

Once BD Matrigel Matrix GFR Phenol Red Free is thawed, swirl vial to be sure that material is evenly dispersed. Handle using sterile technique. Place thawed vial of BD Matrigel Matrix GFR Phenol Red Free in sterile area, spray top of vial with 70% ETOH and air dry. BD Matrigel Matrix GFR Phenol Red Free may be gently pipetted using a pre-cooled pipette to ensure homogeneity.

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

Dispense remaining material into appropriate aliquots, using pre-cooled tubes, and refreeze immediately. Avoid multiple freeze thaws. **DO NOT STORE IN FROST-FREE FREEZER.**

CAUTION:

BD Matrigel Matrix GFR Phenol Red Free will gel rapidly at 22°C to 35°C. Thaw overnight at 4°C on ice (Matrigel may gel at slightly elevated temperatures in a refrigerator). Keep product on ice before use, and use pre-cooled pipettes, tips, and tubes when preparing BD Matrigel Matrix GFR Phenol Red Free for use. Gelled BD Matrigel Matrix GFR Phenol Red Free may be re-liquified if placed at 4°C on ice for 24-48 hours.

COATING PROCEDURES:

BD 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: Some investigators prefer to dilute Matrigel Basement Membrane Matrix. If you wish to maintain a gelled consistency, do not dilute more than 1:3. Use serum-free medium to dilute Matrigel Basement Membrane Matrix. Once gelled, Matrigel Basement Membrane Matrix should be used immediately.

Thin Gel Method

1. Thaw BD Matrigel Matrix GFR Phenol Red Free as recommended. Using cooled pipettes, mix the BD Matrigel Matrix GFR Phenol Red Free to homogeneity.
2. Keeping culture plates on ice, add 50 µL per square centimeter of growth surface.
3. Place plates at 37°C for 30 minutes. Plates are now ready to use.

Thick Gel Method

1. Thaw BD Matrigel Matrix GFR Phenol Red Free as recommended. Using cooled pipettes, mix the BD Matrigel Matrix GFR Phenol Red Free to homogeneity.
2. Keep culture plates on ice. Add cells to BD Matrigel Matrix GFR Phenol Red Free and suspend using cooled pipettes. Add 150-200 µL per square centimeter 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 BD Matrigel Matrix GFR Phenol Red Free as recommended. Using cooled pipettes, mix the BD Matrigel Matrix GFR Phenol Red Free to homogeneity.
2. Dilute BD 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 BD 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:

Dispase (Catalog No. 354235), BD Cell Recovery Solution (Catalog No. 354253)

Most efficient recovery of cells growing on BD Matrigel Matrix GFR Phenol Red Free is accomplished using BD Cell Recovery Solution that depolymerizes the Matrigel Matrix within 7 hours on ice or with Dispase, a metalloenzyme which gently releases the cells allowing for continuous culture.

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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

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

BD Biosciences – Discovery Labware

Two Oak Park, Bedford, MA 01730, tel: 877.232.8995, fax: 800.325.9637, bdbiosciences.com

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