GUIDELINES FOR USE

PRODUCT: BD Matrigel™ Basement Membrane Matrix, 5 mL vial

CATALOG NUMBER: 356234

BACKGROUND: Basement membranes are thin extracellular matrices underlying cells in vivo. BD 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 BD 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. BD 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 BD 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 human19,20 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 vitro26,27 and in vivo,28-30 BD Matrigel Basement Membrane Matrix also supports in vivo propagation of human tumors in immunosuppressed mice.21-33 BD Matrigel Basement Membrane Matrix can be used for the transplantation of unsorted mammary cells,34 as well as sorted epithelial subpopulations embedded in BD 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.
BD 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 BD Matrigel Basement Membrane Matrix is lot specific and can be found on the product Certificate of Analysis.

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

RECONSTITUTION AND USE: Color variations may occur in frozen or thawed vials of BD 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.

Thaw BD Matrigel Basement Membrane Matrix by submerging the vial in ice in a 4°C refrigerator, in the back, overnight. Once BD Matrigel Basement Membrane Matrix is thawed, swirl vial to ensure that material is evenly dispersed. Keep BD Matrigel Matrix on...
ice at all times. Handle with sterile technique. Place thawed vial of BD Matrigel Basement Membrane Matrix in sterile area, spray top of vial with 70% ethanol and air dry.

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

BD 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 BD 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:**
BD 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 BD support web page.* The protein concentration for BD Matrigel Matrix products is lot specific and provided on the Certificate of Analysis. For consistent results dilute BD Matrigel Matrix products by calculating the specific protein concentration (mg/mL) required. To maintain a gelled consistency we recommend not diluting BD Matrigel Matrix to less than 3 mg/mL. Use ice-cold serum-free medium to dilute BD Matrigel Matrix. Mix by pipetting up and down or by swirling the vial in ice.

**Thin Gel Method**

1. Thaw BD Matrigel Basement Membrane Matrix as recommended. Using cooled pipets, mix the BD Matrigel Basement Membrane Matrix 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 BD Matrigel Basement Membrane Matrix as recommended. Using cooled pipets, mix the BD Matrigel Basement Membrane Matrix to homogeneity.
2. Keep culture plates on ice. Add cells to BD Matrigel Basement Membrane Matrix 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 BD Matrigel Basement Membrane Matrix as recommended. Using cooled pipets, mix the BD Matrigel Basement Membrane Matrix to homogeneity.
2. Dilute BD 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 BD 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:
BD™ Dispase (Cat. No. 354235), BD™ Cell Recovery Solution (Cat. No. 354253).

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

*NOTE: For technical resources please visit support page at bdbiosciences.com/cellculture/ecm

REFERENCES:

**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.**