GUIDELINES FOR USE

PRODUCT: Corning® Matrigel® Basement Membrane Matrix High Concentration, 10 ml vial
CATALOG NUMBER: 354248

BACKGROUND:

Basement membranes are thin extracellular matrices underlying cells *in vivo*. Corning Matrigel Matrix High Concentration (HC) 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 HC 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 HC 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 HC will influence gene expression in adult rat hepatocytes[12,13] as well as three dimensional culture in mouse[14-17] 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] Corning Matrigel Matrix HC also supports *in vivo* propagation of human tumors in immunosupressed mice.[30-32] For further information, go to our website at www.corning.com/lifesciences.

SOURCE:

Engelbreth-Holm-Swarm (EHS) Mouse Tumor

FORMULATION:

Dulbecco's Modified Eagle's Medium with 50 µg/ml gentamycin
Corning Matrigel Matrix HC 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:

Color variations may occur in frozen or thawed vials of Corning Matrigel Matrix HC, 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.

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

Corning Matrigel Matrix HC may be used as a thin gel layer (0.5mm), with cells plated on top. Cells may also be cultured inside the Corning Matrigel Matrix HC, 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 HC can be used to assess in vivo angiogenic activity of different compounds by subcutaneous injection into mice (Corning Matrigel Plug Assay).[2,8,25] The high protein

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concentration augments the growth of tumors 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.

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:
Corning Matrigel Matrix HC 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 Corning Matrigel Matrix HC for use. Gelled Corning Matrigel Matrix HC may be re-liquified if placed at 4°C on ice for 24-48 hours.

INJECTION PROTOCOL:

1. It is critical to keep the Corning Matrigel Matrix HC and the Corning Matrigel/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/Cell suspension as asceptic as possible throughout the procedure.

2. For each recipient mouse, mix cells (2×10^5 or greater) and Corning Matrigel Matrix HC 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 µl ice cold medium containing 2×10^6 cells/ml is mixed with 250 µl ice cold Corning Matrigel Matrix HC.

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 Matrigel 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 CLS-DL-CC-036 (Technical Bulletin 455: Methods for Implantation of Corning Matrigel Matrix into Mice and Tissue Fixation).

CELL RECOVERY:
Dispase (Catalog No. 354235), Corning Cell Recovery Solution (Catalog No. 354253)

Most efficient recovery of cells growing on Corning Matrigel Matrix HC is accomplished using Corning 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**

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