

GUIDELINES FOR USE

PRODUCT: Corning® Matrigel® hESC-qualified Matrix, 5 mL vial

CATALOG NUMBER: 354277

BACKGROUND: Basement membranes are continuous sheets of specialized extracellular matrix that are found at the dermal-epidermal junction, at the base of all lumen-lining epithelia throughout the digestive, respiratory, reproductive and urinary tracts and that underlie parenchyma of endocrine and exocrine glands.

Corning Matrigel hESC-qualified Matrix is a soluble basement membrane extract of the Engelbreth-Holm-Swarm (EHS) tumor that gels at room temperature to form a genuine reconstituted basement membrane.¹ The major components of Corning Matrigel hESC-qualified Matrix are laminin, collagen IV, entactin and heparan sulfate proteoglycan.²⁻³ Growth factors, collagenases, plasminogen activators and other undefined components have also been reported in Corning Matrigel hESC-qualified Matrix.^{4,5}

STEM CELLS: Historically, human embryonic stem (hES) cell derivation and culturing techniques utilized serum and/or mouse embryonic fibroblast (MEF) feeder layers.⁶ An ideal environment for hES cell research consists of both a cell culture surface specifically qualified for hES cells, and a serum-free, defined medium. Corning Matrigel hESC-qualified Matrix and STEMCELL Technologies' mTeSR™1 (developed under license from the WiCell Research Institute),⁷ a high quality surface and medium combination, create the first complete environment to support feeder-independent expansion of hES cells.

Corning Matrigel hESC-qualified Matrix is an optimized surface for your stem cell research. It has been qualified as mTeSR1-compatible by STEMCELL Technologies, eliminating the need for time-consuming screening, while providing the reproducibility and consistency essential for your hES cell research. When coupled with a variety of culture media, Corning Matrigel hESC-qualified Matrix has been widely accepted as an alternative substrate for the culture of hES cells as well as human induced pluripotent stem (iPS) cells.⁸⁻¹¹ The mTeSR1 formulation is defined and serum-free, and has been designed to maintain and expand hES cells in an undifferentiated state when used with Corning Matrigel hESC-qualified Matrix as a substrate. It does not require any further addition of growth factors or supplements.

The mTeSR1 formulation and Corning Matrigel hESC-qualified Matrix have been shown to be a successful combination for culturing different hES cell lines for up to 20 passages.¹² Cells maintained in mTeSR1 express high levels of pluripotency markers such as Oct-3/4 and SSEA-3, and pluripotency of cells maintained in mTeSR1 has also been demonstrated by the ability of these cells to differentiate into all three germ layers in the teratoma assay.^{7,13}

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

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)
CLSTechServ@Corning.com www.corning.com/lifesciences

CORNING

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

For a listing of trademarks, visit www.corning.com/lifesciences/trademarks

© 2013 Corning Incorporated

All other trademarks in this document are the property of their respective owners.

FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/mL gentamycin.
Corning® Matrigel® hESC-qualified Matrix is compatible with all culture media.

STORAGE: Stable when stored at -20°C. Store aliquots in either the -20°C or -70°C freezer until ready for use. Freeze thaws should be minimized by aliquotting into one time use aliquots. **DO NOT STORE IN FROST-FREE FREEZER. KEEP FROZEN.**

EXPIRATION DATE: The expiration date for Corning Matrigel hESC-qualified Matrix is lot specific and can be found on the product Certificate of Analysis.

CAUTION: It is extremely important that Corning Matrigel hESC-qualified Matrix and all cultureware or media coming in contact with Corning Matrigel hESC-qualified Matrix should be pre-chilled/ice-cold since Corning Matrigel hESC-qualified Matrix will start to gel above 10°C.

RECONSTITUTION AND USE:

Color variations may occur in frozen or thawed vials of Corning Matrigel hESC-qualified Matrix, ranging from straw yellow to dark red due to the interaction of carbon dioxide with the bicarbonate buffer and phenol red. This is normal, does not affect product efficacy, and will disappear upon equilibration with 5% CO₂.

Thaw Corning Matrigel hESC-qualified Matrix by submerging the vial in ice in a 4°C refrigerator, in the back, overnight. Once Corning Matrigel hESC-qualified Matrix is thawed swirl vial to ensure that material is evenly dispersed. Spray top of vial with 70% ethanol and air dry. Keep product on ice and handle using sterile technique. Dispense material into appropriate aliquots in pre-cooled tubes, switching tips whenever Corning Matrigel hESC-qualified Matrix is clogging the tip and/or causing the pipet to measure inaccurately and refreeze immediately. Gelled Corning Matrigel hESC-qualified Matrix may be re-liquified if placed at 4°C in ice for 24-48 hours.

DILUTION FACTOR: The dilution is calculated for each lot based on the protein concentration. To use with STEMCELL Technologies' mTeSR™1 medium, prepare aliquots according to the dilution factor provided on the Certificate of Analysis. The volume of the aliquots is typically between 270-350 µL.

To Use: Add one aliquot of Corning Matrigel hESC-qualified Matrix to 25 mL of DMEM/F-12 to coat four 6-well plates (1 mL/well) or three 100 mm dishes (8 mL/dish). Incubate the cultureware at room temperature (15-25°C) for at least 1 hour before use. Aspirate the remaining liquid from cultureware just before use. Ensure that the tip of the pipet does not scratch the coated surface. Plates/dishes are now ready to use.

NOTE: For more details on specific applications of Corning Matrigel matrix visit support page at www.corning.com/lifesciences for technical bulletins/application notes, protocols, and frequently asked questions.

REFERENCES:

1. Kleinman HK, et al, Basement membrane complexes with biological activity, *Biochemistry*, **25**:312 (1986).
2. Kleinman HK, et al, Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma, *Biochemistry*, **21**:6188 (1982).
3. Bissell DM, et al, Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver, *J Clin Invest*, **79**(3):801 (1987).
4. Vukicevic S, et al, Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components, *Exp Cell Res*, **202**:1 (1992).
5. McGuire PG, and Seeds NW, The interaction of plasminogen activator with a reconstituted basement membrane matrix and extracellular macromolecules produced by cultured epithelial cells, *J Cell Biochem*, **40**:215 (1989).
6. Thomson JA, et al, Embryonic stem cell lines derived from human blastocysts, *Science*, **282**:1145 (1998).
7. Ludwig TE, et al, Feeder-independent culture of human embryonic stem cells, *Nat Methods*, **3**(8):637 (2006).
8. Xu C, et al, Feeder-free growth of undifferentiated human embryonic stem cells, *Nat Biotechnol*, **19**:971 (2001).
9. Xu C, et al, Immortalized fibroblast-like cells derived from human embryonic stem cells support undifferentiated cell growth, *Stem Cell*, **22**:972 (2004).
10. Drukker M, et al, Isolation of primitive endoderm, mesoderm, vascular endothelial and trophoblast progenitors from human pluripotent stem cells, *Nat Biotechnol.*, **30**(6):531 (2012).
11. Hammerick, KE, et al, Elastic properties of induced pluripotent stem cells, *Tissue Eng Part A*, **17**(3-4):495 (2011).
12. Ludwig TE, et al, Derivation of human embryonic stem cells in defined conditions, *Nat Biotechnol*, **24**:185 (2006).
13. Amit M, et al, Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture, *Dev Biol*, **227**:271 (2000).

California Proposition 65 Notice

WARNING: This product contains a chemical known to the state of California to cause cancer.

Component: **Chloroform**

NOTE: Human embryonic stem cell research may be restricted in your national jurisdiction. Prior to the use of this product for hESC research, please consult your applicable laws regarding such activities.

RELATED PRODUCT: mTeSR™1 Maintenance Medium for Human Embryonic Stem Cells 500 mL (1 kit) Cat. No. **05850**. Please visit www.stemcell.com for more information.

STEMCELL Technologies, Inc.

tel: 800.667.0322, fax: 800.567.2899, e-mail: info@stemcell.com, www.stemcell.com

mTeSR is a trademark of WiCell Research Institute.

To place an order in the U.S., contact Customer Service at:
tel: 800.492.1110, fax: 978.442.2476; email: CLSCustServ@corning.com.

For technical assistance, contact Technical Support at:
tel: 800.492.1110, fax: 978.442.2476; email: CLSTechServ@corning.com.

Outside the U.S., contact your local distributor or visit www.corning.com/lifesciences to locate your nearest Corning office.

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)
CLSTechServ@Corning.com www.corning.com/lifesciences

CORNING

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

For a listing of trademarks, visit www.corning.com/lifesciences/trademarks

© 2013 Corning Incorporated

All other trademarks in this document are the property of their respective owners.