

# CULTREX<sup>®</sup> Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

## 3-D Culture Matrix<sup>™</sup> Laminin I

**Catalog #:** 3446-005-01

**Size:** 5 ml

**Description:** 3-D Culture is an innovative approach to modeling the morphological effects of early oncogenesis on three-dimensional microenvironments. When healthy, differentiating cells exhibit a structured, polarized morphology that is critical for cellular formation and function. During carcinoma development, cell cycle controls associated with cellular development, proliferation and death are lost, and as a result, these structures are disrupted. In effect, the morphology of these structures can be used as a measure to study factors in early carcinoma development. In an attempt at standardization, Debnath, *et al.* published guidelines for execution of this assay using MCF-10A mammary epithelial cells as a model.<sup>1</sup> To aid in the advancement of this technology, Trevigen has developed the Cultrex<sup>®</sup> 3-D Culture Matrix<sup>™</sup> product line to provide reagents specifically produced for and qualified in 3-D culture studies. The 3-D Culture Matrix<sup>™</sup> Laminin I may be used as a gel on which to grow cells or a media additive alone or in concert with other basement membrane components to study cellular growth and differentiation in three dimensions *in vitro*.

To provide the most standardized Laminin I for use in 3-D cultures, a special process is employed to provide material at a standard concentration of approximately 6 mg/ml (by absorbance and extinction coefficient). This material is then incorporated in a 3-D culture to validate efficacy.

### Specifications:

**Concentration:** Please see lot specific data

**Purity:** ≥90% (SDS-PAGE)

**Source:** Murine Engelbreth-Holm-Swarm (EHS) tumor

**Storage Buffer:** Dulbecco's Modified Eagle's medium containing 10 µg/ml gentamycin sulfate and no phenol red.

**Storage/Stability:** Store at -20°C or at -80°C in a manual defrost freezer.

### Materials Qualification:

#### Gelling:

- Laminin I forms a firm gel at neutral pH and 37°C at 6 mg/ml.

#### Functional Assays:

- Cell Attachment: Tested for the ability to promote cell attachment and spreading of MG63 human osteosarcoma cells.
- 3-D Culture: Laminin I promotes attachment and growth of a human epithelial cell line derived from mammary gland (MCF-10A) and human prostate (PC-3), and in the presence of assay medium, these cell lines differentiate into acinar structures.

#### Sterility Testing:

- No bacterial or fungal growth detected after incubation at 37 °C for 14 days following USP XXIV Chapter 71 sterility test.
- No mycoplasma contamination detected by PCR.
- Endotoxin concentrations ≤ 20 EU/ml by LAL assay.

### 3-D Culture Overview:

**Note: This procedure must be conducted in an aseptic environment, such as a laminar flow hood or clean room, using aseptic technique to prevent contamination.**

1. Culture cells as recommended by cell supplier to establish a stable population at 37 °C in a CO<sub>2</sub> incubator; growth media, growth factors, serum requirements, and incubation period may vary by cell type, e.g. MCF-10A (DMEM, 5% Horse Serum, 20 ng/ml hEGF, 500 ng/ml Hydrocortisone, 100 ng/ml Cholera Toxin, 10µg/ml Insulin, 1X Pen/Strep) and PC-3 (RPMI, 10% Horse Serum, 5% Fetal Bovine Serum).
2. Thaw 3-D Culture Matrix Laminin I at 4 °C overnight.
3. Working on ice, add 250 µl of 3-D Culture Matrix Laminin I to each well in a sterile 48 well plate (enough matrix is supplied to assay approximately 20 wells); incubate plate at 37 °C overnight to promote gelling of matrix.
4. Working on ice, add 98 ml of growth media (as recommended by cell supplier) and 2 ml of 3-D Culture Matrix Laminin I or other differentiation factor (final concentration of 2%) to a sterile container, and label this container "Assay Media," and swirl to mix. Any unused Laminin I can be stored at 4 °C up to one week or stored in working aliquots at -20 °C in a manual defrost freezer.
5. Incubate Assay Media at 37 °C for 30 minutes in preparation for cell dilution.
6. Harvest cells from culture, and dilute cells to 1 x 10<sup>4</sup> cells/ml in 25 ml (total volume) of Assay Medium.
7. Add 500 µl of cell suspension to each well of the 48 well plate containing 3-D Culture Matrix Laminin I (5,000 cells/well).
8. Incubate plate at 37 °C in a CO<sub>2</sub> incubator.
9. Each day, observe cell growth and structure formation via inverted microscope.
10. On day 4, carefully pipette off old media using a sterile serological pipette, and replace with new Assay Media. Repeat on day 8 and day 12.
11. When structures have grown to desired size, prepare cells for analysis (as recommended by manufacturer), and analyze structures. This point is dependent on cell line and growth conditions. In our qualification, MCF-10A cells are analyzed at 16 days, and PC-3 cells are analyzed at 10 to 12 days.

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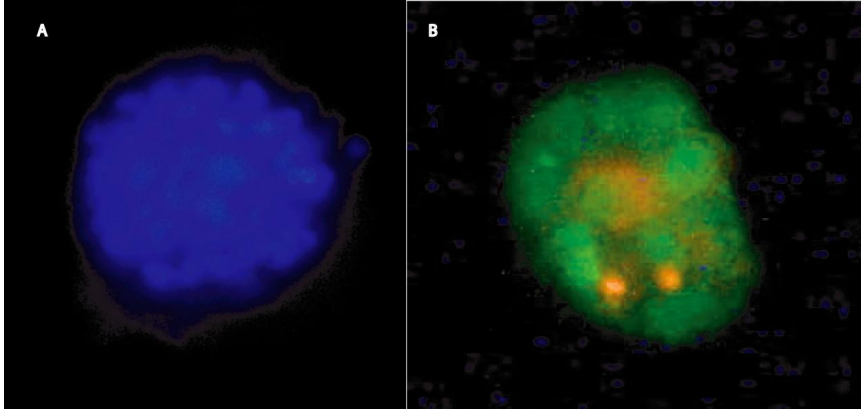
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Recommendations for analysis:

1. To fix cells, incubate for 20 minutes in 2% formalin, 1X PBS at room temperature.
2. Cells may be analyzed in the plate on Laminin I; they may be transferred to a microscope slide (very carefully); or they may be embedded in paraffin and sectioned.
3. Cells may also be isolated from the Laminin I and processed for protein, DNA or RNA analysis.



3-D Culture of MCF-10A Breast Cancer Cells using 3-D Culture Matrix™ Laminin I for 15 days in the presence of 2% Laminin I in Assay Medium, and stained using A) CPA Dye 2, and B) SYBR® Green (nuclear) and propidium iodide (dead or necrotic).

**References:**

1. Debnath J, Muthuswamy SK, Brugge JS. Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods*, 2003 pp. 256-268.
2. Fridman R, Giaccone G, Kanemoto T, Martin G, Gazdar A, and Mulshine J. Reconstituted basement membrane (matrigel) and laminin can enhance the tumorigenicity and the drug resistance of small cell lung cancer cell lines. *Proc. Natl. Acad. Sci.* 1990. 87:6698-6702.
3. Kubota Y, Kleinman H, Martin G, and Lawley T. Role of laminin and basement membrane proteins in the morphological differentiation of human endothelial cells in capillary-like structures. *J. Cell Biol.* 1988. 107:1589-1598.
4. Ponce M., Nomizu M, Delgado M, Kuratomi V, Hoffman M, Powell S, Yamada Y, Kleinman H, and Malinda K. Identification of endothelial cell binding sites on the laminin g1 chain. *Circ. Res.* 1999. 84:688-694.
5. Taub, M, Wang Y, Szczesny T, and Kleinman H. Epidermal growth factor or transforming growth factor a is required for kidney tubulogenesis in matrigel cultures in serum-free medium. *Proc. Natl. Acad. Sci.* 1990. USA 87:4002-4006.
6. Lang SH, Sharrard RM, Stark M, Villette JM, and Maitland NJ. Prostate epithelial cell lines form spheroids with evidence of glandular differentiation in three-dimensional Matrigel cultures. *Br J Cancer.* 2001 Aug 17; 85(4): pp. 590-599.
7. Webber MM, Bello D, Kleinman HK, and Hoffman MP. Acinar differentiation by non-malignant immortalized human prostate epithelial cells and its loss in malignant cells. *Carcinogenesis.* 1997. Jun; 18(6): 1225-1231.
8. Fong CJ, Sherwood ER, Sutkowski DM, Abu-Jawdeh GM, Yokoo H, Bauer KD, Kozlowski JM, and Lee C. Reconstituted basement membrane promotes morphological and functional differentiation of primary human prostate epithelial cells. *Prostate.* 1991; 19(3): 221-235.
9. U.S. Patent 4,829,000
10. U.S. Patent 5,158,874

Lot Specific Data

Lot Number:  
Protein Concentration:  
Endotoxin (LAL):



**Mouse Laminin I**  
Catalog #: 3446-005-01  
Storage: -20 °C  
(Manual Defrost Freezer)  
1-800-873-8443