Cultrex® 3-D Culture Matrix Laminin I

Catalog Number: 3446-005-01 (30 mg)

PRODUCT 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. The 3-D Culture Matrix Rat Collagen was designed to 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* (1-10).

Cultrex 3-D Culture Matrix Laminin I is a purified basement membrane protein that has been developed, produced and qualified specifically for use in 3-D culture studies. 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.

INTENDED USE

Cultrex 3-D Culture Matrix Laminin I is an extracellular matrix hydrogel that directs cells to grow in three dimensions and assemble into organotypic structures *in vitro*. It may also be used as to supplement customized hydrogel or medium formulations for cell culture.

PRODUCT SPECIFICATIONS

Concentration	6 mg/mL
Source	Murine Engelbreth-Holm-Swarm (EHS) tumor.
Storage Buffer	Dulbecco's Modified Eagle's Medium without phenol red, containing 10 µg/mL gentamicin sulfate.
Stability	Product is stable for a minimum of 3 months from date of shipment. See lot specific Certificate of Analysis for expiration date.
Storage	Store at ≤ -20 °C in a manual defrost freezer. For optimal stability, store at ≤ -70 °C. **Avoid freeze-thaw cycles.**

PRECAUTION

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- Results may vary due to variations among tissue/cells derived from different donors or sources.

MATERIAL QUALIFICATIONS

Sterility Testing:

- Tested following USP <71> sterility guidelines.
- No mycoplasma contamination detected by PCR.
- Endotoxin concentrations ≤ 20 EU/mL by LAL assay.

Functional Assays:

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

Gelling Assay:

- Cultrex 3-D Culture Matrix Laminin I forms a firm gel at neutral pH and 37 °C at 6 mg/mL.

3-D CULTURE PROCEDURE:

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_2 incubator; growth media, growth factors, serum requirements, and incubation period may vary by cell type. Two examples are provided below:
   a. MCF-10A culture medium contains DMEM, 5% Horse Serum, 20 ng/mL hEGF, 500 ng/mL Hydrocortisone, 100 ng/mL Cholera Toxin, 10 µg/mL Insulin, and 1X Pen/Strep.
   b. PC-3 culture medium contains: RPMI, 10% Horse Serum, 5% Fetal Bovine Serum.
2. Thaw Cultrex 3-D Culture Matrix Laminin I at 2-8 °C overnight.
3. Working on ice, add 250 µL of Cultrex 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 Cultrex 3-D Culture Matrix Laminin I or other differentiation factor (final concentration of 2%) to a sterile container, and label this container "Assay Media". Swirl to mix. Any unused Cultrex 3-D Culture Matrix Laminin I can be stored at 2-8 °C up to one week or stored in working aliquots at ≤ -20 °C in a manual defrost freezer.
5. Incubate Assay Medium at 37 °C for 30 minutes in preparation for cell dilution.
6. Harvest cells from culture, and dilute cells to 1 x 10^4 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 already containing Cultrex 3-D Culture Matrix Laminin I (5,000 cells/well).
8. Incubate plate at 37 °C in a CO_2 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 Medium. Repeat on day 8 and day 12.
11. When structures have grown to desired size, prepare cells for analysis 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.

3-D CULTURE FIXATION AND ANALYSIS:

1. To fix cells, incubate for 20 minutes in 2% Formalin in 1X PBS at room temperature.
2. Cells may be analyzed in the plate, they may be carefully transferred to a microscope slide, 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.

REFERENCES

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