PRODUCT DESCRIPTION

3-D culture methods provide cells with the necessary structure and signaling cues for the reconstruction of native tissue architecture, providing more physiologically predictive in vitro models for evaluating development and disease. In 3-D culture conditions, normal cells can assemble into organoids, which structurally resemble their tissue of origin, exhibit a polarized morphology, undergo cell cycle regulation, and produce tissue-specific proteins. Cancer cells grown in 3-D culture conditions assemble into tumor-like structures, lacking an organized architecture or cell cycle regulation, and exhibiting tumor-specific markers depending on the extent of malignancy.

Cultrex Basement Membrane Extract (BME) is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumor. Cultrex BME gels at 37 °C to form a reconstituted basement membrane. The major components of Cultrex BME include laminin, collagen IV, entactin, and heparin sulfate proteoglycan.

INTENDED USE

Cultrex 3-D Culture Matrix Reduced Factor Basement Membrane Extract (RGF BME) is produced and qualified specifically for use in 3-D culture studies. Cultrex 3-D Culture Matrix RGF BME provides the foundation for cells to grow in three dimensions allowing for the formation of complex in vitro structures. To provide the most standardized basement membrane extract for use in 3-D cultures, a special process is employed to reduce growth factors and manufacture matrix at a standard and consistent concentration. This product is then evaluated in 3-D culture assays to validate efficacy.

PRODUCT SPECIFICATIONS

<table>
<thead>
<tr>
<th>Specification</th>
<th>Details</th>
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<tbody>
<tr>
<td>Concentration</td>
<td>8-12 mg/mL as determined by Lowry assay.</td>
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<tr>
<td>Source</td>
<td>Murine Engelbreth-Holm-Swarm (EHS) tumor.</td>
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<tr>
<td>Storage Buffer</td>
<td>Dulbecco’s Modified Eagle’s Medium without phenol red, containing 10 μg/mL gentamicin sulfate.</td>
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<tr>
<td>Stability</td>
<td>Product is stable for two years from date of manufacture. See lot specific Certificate of Analysis for expiration date.</td>
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<tr>
<td>Storage</td>
<td>Store at ≤ -70 °C. Product may be thawed and dispensed into working aliquots. Avoid freeze-thaw cycles.</td>
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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:

• PathClear - Tested negative by PCR test for 31 organisms and viruses, including: mycoplasma, 17 bacterial and virus strains typically included in mouse antibody production (MAP) testing, and 13 additional murine infectious agents including LDEV.
• Tested following USP <71> sterility guidelines.
• Endotoxin concentration ≤ 8 EU/mL by LAL assay.

Functional Assays:

• 3-D culture: Cultrex 3-D Culture Matrix RGF BME promotes differentiation of a human epithelial cell line derived from mammary gland (MCF-10A) or human prostate (PC-3) into acinar structures.
• Tube formation assay - Cultrex 3-D Culture Matrix RGF BME promotes formation of capillary-like structures by human (HBMVEC, HUVEC) or mouse (SVEC4-10) endothelial cells.

Gelling Assay

• Cultrex 3-D Culture Matrix RGF BME gels in less than 30 minutes at 37 °C, and maintains the gelled form in culture medium for a minimum of 14 days at 37 °C.

3-D CULTURE PROCEDURE

This protocol is modified from Debnath, J. et al. (2003) Methods 30:256. Different cell lines may require different cell culture conditions and incubation periods.

1. Culture cells as recommended by cell supplier to establish a stable population at 37 °C in a CO₂ incubator; growth media, growth factors, serum requirements, and incubation period may vary by cell type (i.e. MCF-10A; DMEM, 5% Horse Serum (HS), 20 ng/mL hEGF, 500 ng/mL Hydrocortisone, 100 ng/mL Cholera Toxin, 10 μg/mL Insulin, 1X Pen/Strep; and PC-3: RPMI-1640, 10% HS, 5% Fetal Bovine Serum (FBS)).
2. Thaw 3-D Culture Matrix RGF BME at 2-8 °C overnight.
3. Working on ice, add 250 μL of Cultrex 3-D Culture Matrix RGF BME per well of a sterile 48-well plate, incubate the plate at 37 °C for 30 minutes to promote gelling of matrix.
4. Working on ice and using a sterile container, dilute Cultrex 3-D Culture Matrix RGF BME 1:50 in cell culture media to generate Assay Media, a 2% 3-D Culture Matrix RGF BME solution (i.e. add 0.5 mL of Cultrex 3-D Culture Matrix RGF BME to 24.5 mL of cell culture media which accommodates one 48-well plate). Swirl to mix Assay Media. Any unused Cultrex 3-D Culture Matrix RGF BME 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 Media at 37 °C for 30 minutes in preparation for cell dilution.
6. Harvest cells from culture, and dilute cells to 1x10⁶ cells/mL in 24 mL of Assay Media.
7. Add 500 μL of cell suspension to each well of the 48-well plate containing Cultrex 3-D Culture Matrix RGF BME.
8. Incubate plate at 37 °C in a CO₂ incubator overnight.
9. Each day, observe cell growth and structure formation via inverted microscope, and replace 48-well in a CO₂ incubator overnight at 37 °C.
10. On day 4, carefully remove 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.
   Note: Time of analysis 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 10-12 days.

Recommendations for analysis:

12. For fixation of 3-D cell cultures, incubate for 20 minutes in 2% formalin in 1X Phosphate Buffered Saline (PBS) at room temperature.
13. Cells may be analyzed in the plate while still on the Cultrex BME, after careful transition to a microscope slide, or after being embedded in paraffin and sectioned.
REFERENCES