CULTREX® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

3-D Culture Matrix™ Basement Membrane Extract Reduced Growth Factor (phenol red free)

Catalog #s: 3445-005-01 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, J. Debnath, et al. published guidelines for execution of this assay using MCF-10A mammary epithelial cells as a model.[1] To aid in the advancement of this technology, Trevigen has developed Cultrex® 3-D Culture Matrix™ RGF BME which is the first Basement Membrane Extract produced and qualified specifically for use in 3-D culture studies. The 3-D Culture Matrix™ RGF BME provides the foundation for cells to grow in three dimensions allowing for the formation of structures in vitro. To provide the most standardized basement membrane extract for use in 3-D cultures, a special process is employed to reduce growth factors (see table below) and provide material at a standard concentration of approximately 14 mg/mL. This material is then incorporated in a 3-D culture to validate efficacy.

Specifications:

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: Product is stable for a minimum of 3 months from date of shipment when stored at –20 °C in a manual defrost freezer. **For optimal stability, store at –80 °C in aliquots. Keep Frozen; repeated freeze-thaws will destroy product integrity.**

Material Qualification:

Gelling: Basement Membrane Extract 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.

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.

E11/2/09v1

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Functional Assays:

- Tube Assay: Basement Membrane Extract promotes differentiation of a mouse endothelial cell line derived from axillary lymph node (SVEC4-10) into capillary-like structures.
- 3-D Culture: Basement Membrane Extract promotes differentiation of a human epithelial cell line derived from mammary gland (MCF-10A) and human prostate (PC-3) into acinar structures.

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_2 incubator; growth media, growth factors, serum requirements, and incubation period may vary by cell type, e.g. 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, 10% HS, 5% Fetal Bovine Serum (FBS)).
2. Thaw 3-D Culture Matrix RGF BME at 4°C overnight.
3. Working on ice, add 250 µL of 3-D Culture Matrix RGF BME to each well in a sterile 48 well plate (enough matrix is supplied to assay one 48 well plate); incubate plate at 37°C for 30 minutes 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 RGF BME (final concentration of 2%) to a sterile container, and label this container "Assay Media," and swirl to mix. Any unused RGF BME 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 10^4 cells/mL in 24 mL of Assay Medium.
7. Add 500 µL of cell suspension to each well of the 48 well plate containing 3-D Culture Matrix RGF BME.
8. Incubate plate at 37°C in a CO_2 incubator overnight.
9. Each day, observe cell growth and structure formation via inverted microscope, and replace 48 well in a CO_2 incubator overnight at 37°C.
10. On day 4, carefully pipet 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.

Recommendations for analysis:

12. To fix cells, incubate for 20 minutes in 2% formalin, 1X PBS at room temperature.
13. Cells may be analyzed in the plate on BME; they may be transferred to a microscope slide (very carefully); or they may be embedded in paraffin and sectioned.

References:

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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.
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6. Lang SH, Sharrard RM, Stark M, Villette JM, and Maitland NJ. Prostate epitheial cell lines form spheroids with evidence of glandular differentiation in three-dimensional Matrigel cultures. Br J Cancer. 2001 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. 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

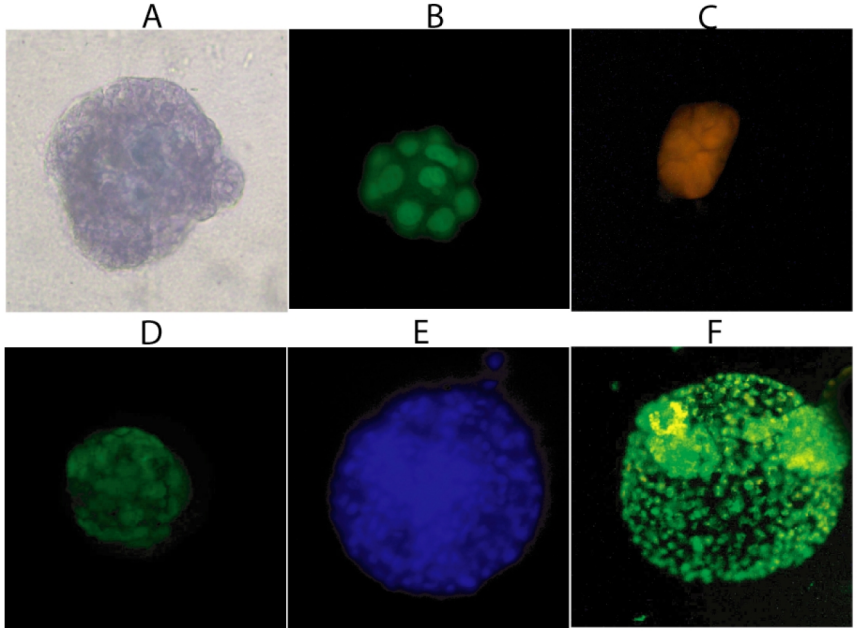

Figure 1. Three-Dimensional Cellular Structures. Staining of MCF-10A cells after sixteen days in 3-D Culture Matrix™ RGF BME with: A) Cell Staining Kit (structural), B) SYBR® Green (nuclear), and C) MitoShift™ (mitochondrial potential); and staining of PC-3 cells after twelve days in 3-D Culture Matrix™ RGF BME with: D) Calcein AM (cell viability), E) CPA dye 1 (nuclear) and F) Depsipher™ (mitochondrial potential).

Related Products:

Catalog#	Description	Size
3446-005-01	Cultrex® 3-D Culture Laminin I	5 ml
3447-020-01	Cultrex® 3-D Collagen I Rat Tail	100 mg
3445-005-01	Cultrex® 3D Culture 96 Well BME Cell Proliferation Assay	96 tests
3446-096-K	Cultrex® 3D Culture 96 Well Laminin I Cell Proliferation Assay	96 tests
3447-096-K	Cultrex® 3D Culture 96 Well Collagen I Cell Proliferation Assay	96 tests
3448-020-K	Cultrex® 3D Culture Cell Harvesting Kit	20 tests

Reduced Growth Factor
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