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CULTREX® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

3-D Culture Matrix™ BME Coated 96 Well Plate

Catalog #: 3445-096-CP Size: Each

Description: 3-D Culture is an innovative approach to modeling the morphological effects of oncogenesis and development using three-dimensional microenvironments. Extracellular matrix proteins form hydrogels under physiological conditions that mimic the cell environment *in vivo*, and as a result, these cells assume structural and functional characteristics of their emanating tissues. The 3-D Culture Matrix BME Coated 96 Well Plate is coated with a reconstituted basement membrane matrix to provide the environment that is common for epithelial or endothelial cell types, and this plate provides a convenient, standardized, physiologically predictive format for evaluating the pharmacological effects of compounds for these cell culture models.

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. Keep Frozen; repeated freeze-thaws will destroy product integrity.**

Material Qualification:

Gelling: Basement Membrane Extract forms a hydrogel that supports cell proliferation, 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.

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.

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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
2. Thaw 3-D Culture Matrix BME Coated 96 Well Plate at Room Temperature for one hour, and then transfer to a 37 °C cell culture incubator for 30 minutes.
3. Harvest cells from culture, and dilute cells to as needed in 3-D Culture Medium. Optimal cell seeding concentrations are cell line dependent and may need to be determined empirically. Also, please contact the cell supplier or consult the literature to determine what growth factors, cytokines, or other ECM proteins may be needed to support your cell model. Cells may also be treated with compounds during seeding if assessing the impact on proliferation.
7. Add 100 µl of cell suspension to each well of the 96 well plate containing 3-D Culture Matrix BME.
8. Incubate plate at 37°C in a CO_2 incubator, and visually monitor cell growth and morphology. If assessing viability, cells may be treated once physiological structures are formed.

Recommendations for analysis:

9. Cell number may be determined using 3D Culture Proliferation Core Kit (cat# 3445-096-CK).

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.
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5. Taub, M, Wang Y, Szczesny T, and Kleinman H. Epidermal growth factor or transforming growth factor α 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 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

This product is made and marketed under patent license from the United States Public Health Service. Ref. U.S. Patent 4,829,000 issued May 9, 1989 and U.S. Patent 5,158,874 issued October 27,1992, all entitled Reconstituted Membrane Complex with Biological Activity.

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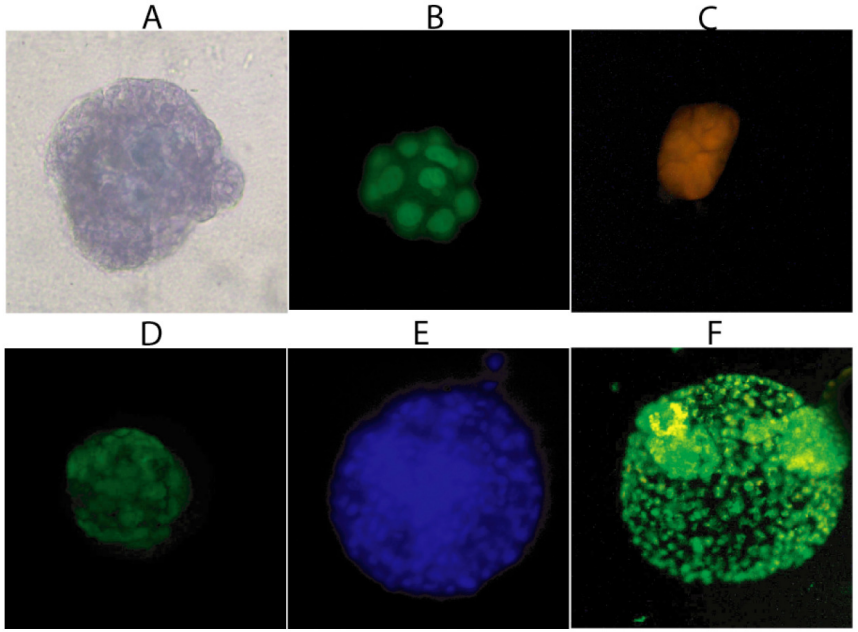

Figure 1. Three-Dimensional Cellular Structures. Staining of MCF-10A cells after sixteen days in 3-D Culture Matrix™ RGF BME with: A) Cell Staining Kit (structural), B) SYBR® Green (nuclear), and C) MitoShift™ (mitochondrial potential); and staining of PC-3 cells after twelve days in 3-D Culture Matrix™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

SYBR® Green is a registered trademark of Molecular Probes, Eugene, OR.
Depsipher and MitoShift are trademarks of Trevigen, Inc.

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
3445-096-K	Cultrex® 3D Culture 96 Well BME Cell Proliferation Assay	96 tests
3445-096-CK	Cultrex® 3D Culture 96 Well Cell Proliferation Assay Core Kit	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

3-D Culture Matrix™ BME Coated
96 Well Plate
Catalog # 3445-096-K
Storage: ≤ -20 °C (Manual Defrost)
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