R&D SYSTEMS
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Cultrex® 3-D Culture Matrix Reduced Growth Factor Basement Membrane Extract, PathClear®

Catalog Numbers: 3445-001-01 (1.0 mL)
3445-005-01 (5.0 mL)
3445-010-01 (2 x 5.0 mL)

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

Concentration	8-12 mg/mL as determined by Lowry assay.
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 two years from date of manufacture. See lot specific Certificate of Analysis for expiration date.
Storage	Store at ≤ -70 °C. Product may be thawed and dispensed into working aliquots. **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:

- 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 CO_2 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 $1x10^4$ 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 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 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

1. Debnath, J. *et al.* (2003) Methods **30**:256.
2. Webber, M.M. *et al.* (1997) Carcinogenesis **18**:1225.
3. Fong, C. J. *et al.* (1991) Prostate **19**:221.
4. Lang, S.H. *et al.* (2001) Br. J. Cancer **85**:590.
5. Taub, M. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:4002.

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