Cultrex® Basement Membrane Extract, Type 3, PathClear® *with Phenol Red*

Catalog Number:	Size:
3632-005-02P	5 mL
3632-010-02P	2 x 5 mL

PRODUCT DESCRIPTION

Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between endothelial, epithelial, muscle, or neuronal cells and their adjacent stroma. Basement membranes are degraded and regenerated during development and wound healing. They not only support cells and cell layers, but they also play an essential role in tissue organization that affects cell adhesion, migration, proliferation, and differentiation. Basement membranes provide major barriers to invasion by metastatic tumor cells.

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 BME include laminin, collagen IV, entactin, and heparin sulfate proteoglycan.

INTENDED USE

Cultrex BME, Type 3 with Phenol Red provides a proprietary formulation that is physiologically aligned with the *in vivo* solid tumor environment and is recommended for xenografts and other *in vivo* applications. The 100X Phenol Red Solution provided can be added to Cultrex BME, Type 3 to monitor pH. Addition of phenol red is optional.

MATERIALS PROVIDED

CATALOG #	PART	PART #	AMOUNT PROVIDED	STORAGE
3632-005-02P	**Cultrex Basement Membrane Extract, Type 3, PathClear**	3632-005-02	5 mL	Store at ≤ -70 °C.
	100X Phenol Red Solution	3430-50-01	50 µL	Store at ≤ -20 °C.
3632-010-02P	**Cultrex Basement Membrane Extract, Type 3, PathClear**	3632-005-02	2 vials (5 mL/vial)	Store at ≤ -70 °C.
	100X Phenol Red Solution	3430-50-01	2 vials (50 µL/vial)	Store at ≤ -20 °C.

PRODUCT SPECIFICATIONS

Cultrex BME, Type 3

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.**

100X Phenol Red Solution

Concentration	1.5 mg/mL
Size	50 µL
Storage Buffer	Dulbecco's Modified Eagle's Medium, with 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 ≤ -20 °C in a manual defrost freezer. **Avoid freeze-thaw cycles.**

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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:

- Tumor Growth Assay - Cultrex BME, Type 3 supports proliferation and growth of breast cancer cells (MCF7) embedded in the matrix for minimum of 8 days.

Gelling Assay:

- Cultrex BME, Type 3 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.

COATING PROCEDURE

Thaw Cultrex BME, Type 3 overnight at 2-8 °C. Refrigerator temperatures may vary, therefore it is recommended to keep BME on ice in a refrigerator during the thawing process. Thawed BME solidifies quickly at temperatures above 15 °C; when working with BME, keep it on ice to prevent untimely gelling.

There are many applications for Cultrex BME, Type 3 which require different thicknesses and concentrations. A thick gel is needed for applications such as endothelial cell formation of capillary-like structures (Tube Formation Assay), the differentiation of rat aorta tissue into capillary-like structures (Aortic Ring Assay), epithelial organoid formation, or tumor organoid formation. Some applications, such as propagation of primary cells, require a thin layer coating and not a thick gel; therefore, the thin layer method should be used.

Thick Gel Method:

1. Thaw Cultrex BME, Type 3 as stated above.
2. Mix Cultrex BME, Type 3 by slowly pipetting solution up and down; be careful not to introduce air bubbles.
   Note: *If incorporating phenol red, dilute 100X Phenol Red Solution to 1X in your desired volume of Cultrex BME, Type 3.*
3. Pipette 200-300 µL per cm^2 onto the growth surface.
4. Place coated object at 37 °C for 30 minutes.
5. Coated objects are ready for use.

Thin Layer Method (non-gelling):

1. Thaw Cultrex BME, Type 3 as stated above.
2. Mix Cultrex BME, Type 3 by slowly pipetting solution up and down; be careful not to introduce air bubbles.
   Note: *If incorporating phenol red, dilute 100X Phenol Red Solution to 1X in your desired volume of Cultrex BME, Type 3.*
3. Dilute Cultrex BME, Type 3 to desired concentration in **cold** serum-free medium. A 1:100 dilution is recommended starting concentration for the propagation of primary cells. Empirical determination of the optimal coating concentration for your application may be required.
4. Add a sufficient amount of solution to cover the entire growth surface area. A volume of 300 µL per cm^2 is recommended.
5. Incubate coated object at room temperature for one hour.
6. Aspirate coating solution and immediately plate cells. **Do not allow coated surface to dry out.**

REFERENCES

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