Basement Membrane Extract
Reduced Growth Factor
(phenol red free)
Catalog #: 3433-005-01
Storage: ≤ -20˚C

TREVIGEN®

1-800-873-8443

コスモ・バイオ株式会社

TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures.

Cultrex® Basement Membrane Extract Reduced Growth Factor (phenol red free)

Catalog #: 3433-005-01

Size: 5 ml
Concentration: ~8 mg/ml

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 repair. 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 is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumor. The extract gels at 37˚C to form a reconstituted basement membrane. The major components of the Basement Membrane Extract include laminin, collagen IV, entactin, and heparin sulfate proteoglycan. The Basement Membrane Extract can be used for promotion and maintenance of a differentiated phenotype in a variety of cell cultures including primary epithelial cells, endothelial cells, and smooth muscle cells. It has been employed in angiogenesis assays, neurite outgrowth assays, tumor cell invasion assays, and as a vehicle to augment the tumorigenicity of injected tumor cells in nude mice.

The reduction in specific growth factor concentrations (see table below) allows use of the Basement Membrane Extract (Reduced Growth Factor) to examine the effects of specific growth factors in applications requiring Basement Membrane Extract.

	Concentrations	
Growth Factor	Basement Membrane Extract (standard)	Basement Membrane Extract (Reduced Growth Factor)
bFGF	0-0.1 pg/ml	0-0.1 pg/ml
EGF	0.5-1.3 ng/ml	< 0.5 ng/ml
IGF-1	15.6 ng/ml	5.0 ng/ml
PDGF	12 pg/ml	< 5.0 pg/ml
NGF	< 0.2 ng/ml	< 0.2 ng/ml
TGF-beta	2.3 ng/ml	1.7 ng/ml

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 Conditions: Store at -20˚C or at -80˚C in a manual defrost freezer.

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Specifications:
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.

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.
- Ring Assay: Basement Membrane Extract promotes differentiation of mouse aorta tissue to form capillary-like structures.

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.

Coating Procedures: Cultrex® Basement Membrane Extract gels rapidly at 22-35˚C. Thaw extract at 2-8˚C overnight. Refrigerator temperatures may vary; therefore, thaw extract on ice in a refrigerator. Prechill pipette tips, tubes, plates, or any other object that may come in contact with the extract to prevent gelling.

There are many applications for Cultrex Basement Membrane Extract, which require different thicknesses and concentrations. In general, Basement Membrane Extract, at a protein concentration ≥ 5 mg/ml, is used for differentiation studies of primary cells. Extract diluted below 5 mg/ml does not form a gel, and will only support the propagation of primary cells, but not their differentiation. For applications such as endothelial cell differentiation into capillary-like structures (Tube Assay) a thin gel is needed. For applications such as the differentiation of rat aorta tissue into capillary-like structures (Ring Assay), or cell invasion assays, a thick gel is needed. Some applications, such as propagation of primary cells, only need a protein layer and not a protein matrix; therefore, the layer method should be used.

Thin Gel Method:
1. Thaw Cultrex Basement Membrane Extract as stated above.
2. Mix extract by triturating solution with prechilled pipettes.
3. Place 50 µl per cm^2 onto a prechilled growth surface
4. Place coated object at 37˚C for 30 minutes.
5. Coated objects are ready for use.

Thick Gel Method:
1. Thaw Cultrex Basement Membrane Extract as stated above.
2. Mix extract by triturating solution with prechilled pipettes.
3. Place 150-200 µl per cm^2 onto a prechilled 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 Basement Membrane Extract as stated above.
2. Mix extract by triturating solution with prechilled pipettes.
3. Dilute the extract to desired concentration in cold serum-free medium. Empirical determination of the optimal coating concentration for your application may be required. A protein concentration of 0.1 mg/ml is a recommended starting concentration for the propagation of primary cells.

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Thin Layer Method (non-gelling):

1. Thaw BME as stated above.
2. Mix extract by triturating solution with a pipette.
3. Dilute the extract to desired concentration in cold serum-free medium. Empirical determination of the optimal coating concentration for your application may be required. A protein concentration of 0.1 mg/ml is a recommended starting concentration for the propagation of primary cells.
4. Add a sufficient amount of solution to cover the entire area onto growth surface.
5. Place coated object at 37˚C for 60 minutes or until dry.
6. Coated objects are ready for use.

In vivo Method:

To minimize absorption of BME into the mouse tissue before gelling occurs it must be placed at room temperature (18-22˚C) for 10-15 minutes.

1. Thaw the BME as stated above.
2. Determine how much BME is needed for your application.
3. Mix extract by triturating solution with a pipette.
4. Mix cells and/or angiogenic compounds at the proper concentrations into the BME.
5. Fill a syringe with the BME.
6. Incubate the syringe at room temperature (18-22˚C) for a total of 15 minutes from the start of this procedure. **Caution: Do not exceed 15 minutes. The BME may gel in the syringe, and prevent the injection into mice.**
7. Inject the volume needed for your particlular protocol. The pre-incubation at room temperature will cause the BME to gel instantly at 37˚C, which will prevent absorption into the mouse tissue.

References:

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Cat# 3433-005-01 v31113