



Cultrex® Stem Cell Qualified Human BME, *PathClear®

Catalog #: 3415-001-03

Size: 1 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® Human Basement Membrane Extract (BME) is a soluble form of basement membrane purified from human placenta. BME can be used for promotion and maintenance of an undifferentiated phenotype (fig. 1), or for the directed differentiation of precursor cells, under a variety of cell culture conditions, including stem cells, primary epithelial cells, endothelial cells and smooth muscle cells.

Specifications:

Concentration: 1 mg/ml

Source: Human Placenta

Storage Buffer: Dulbecco's Modified Eagle's medium containing 10 µg/ml gentamicin 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. For optimal stability store at -80°C. **Repeated freeze-thaws will destroy product integrity.**

Materials Qualification:

Functional Assay:

- Promotes the attachment of H9 human embryonic stem cells.
- Effectively maintains human embryonic stem cells in a pluripotent state as evidenced by intracellular staining for the stem cell markers Oct-4 and Nanog.

*Sterility Testing:

- No bacterial or fungal growth detected after incubation at 37°C for 14 days following USP sterility testing guidelines.
- No mycoplasma contamination detected by PCR.
- Stem Cell Qualified Human BME, PathClear® is tested negative by PCR for different human pathogenic viruses including EBV, HAdV, Hantaan, HCMV, Hepatitis A, Hepatitis B, Hepatitis C, HHV 6, HHV 8, HIV1, HIV2, HSV 1, HSV 2, HTLV 1, HTLV 2, LCMV, Seoul, Sin Nombre, VZV.
- Endotoxin concentration < 20 EU/ml by LAL assay.

Safety Statement: Cultrex® Human BME, PathClear® contains human source material and therefore should be treated as potentially infectious and handled at Biological Safety Level 2 to minimize exposure.

Coating procedure for Stem Cell Propagation:

The recommended working concentration is $10 \mu\text{g}/\text{cm}^2$ of growth surface depending on cell type. Empirical determination of the optimal coating concentration for your application may be required.

1. Thaw Human BME on ice for several hours.
2. In a laminar flow hood, dilute to a final concentration $100 \mu\text{g}/\text{ml}$ with serum-free cell culture medium.
3. Mix and transfer to the wells of tissue culture plates. Spread the solution to completely cover the bottom of the wells.
4. Incubate coated object at room temperature for an hour.
5. Aspirate coating solution and immediately plate cells. **Do not allow coated surface to dry out.**

The following table is a guide for the suggested volumes required per well:

| <u>Plate type/</u> | <u>Volume Human BME per Well</u> |
|-------------------------|----------------------------------|
| 6 wells (or 35 mm dish) | 1 – 1.5 ml |
| 12 wells | 500 - 600 μl |
| 24 wells | 250 – 300 μl |
| 48 wells | 150 μl |
| 96 wells | 50 μl |

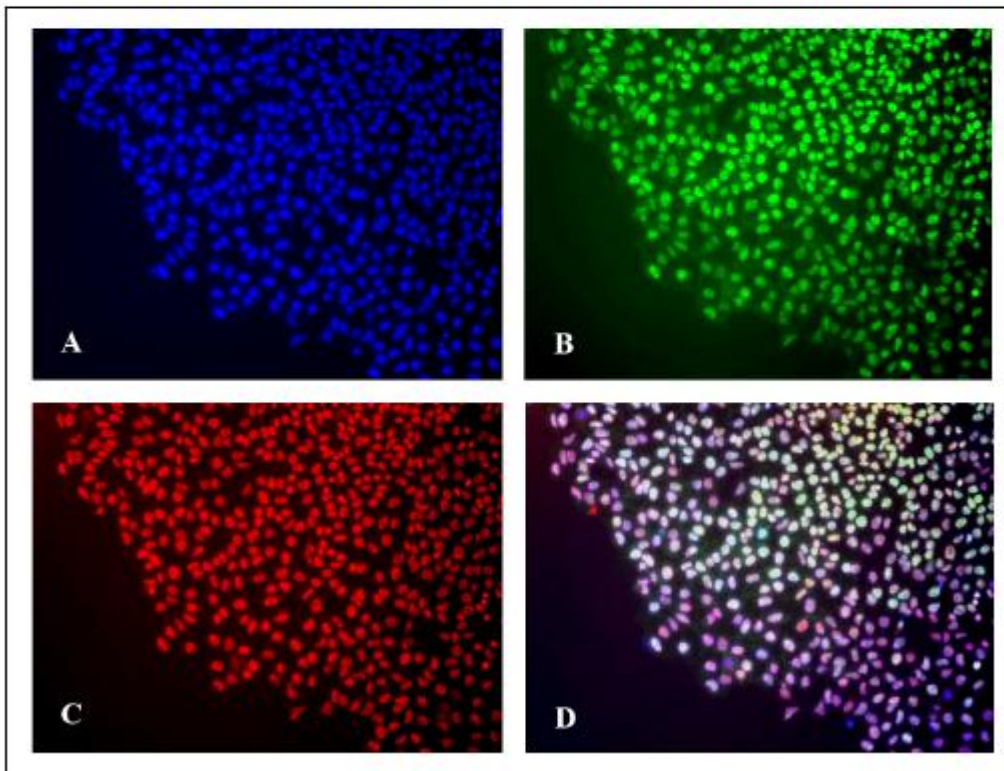


Fig.1. H9 human embryonic stem cells after three passages on Cultrex[®] Stem Cell Qualified Human BME, PathClear[®] maintain expression of the non-differentiated stem cell markers Oct-4 (B) and Sox 2 (C). Nuclear staining by DAPI shown on panel (A) and merged image shown on panel (D).

References:

1. Bilozur, M.E., and E.D. Hay. 1988. Neural crest cell migration in 3 dimensional matrix utilizes laminin, fibronectin or collagen. *Developments in Biologicals* **125**:19-33.
2. Arnaoutova, I., George, J., Kleinman, H.K., and G. Benton. 2011. Basement membrane matrix (BME) has multiple uses with stem cells. *Stem Cell Rev and Rep*, in press.
3. Angel, M., and M.F. Yanik. 2010. Innate immune suppression enables frequent transfection with RNA encoding reprogramming proteins. *PLoS ONE* **5**: e11756.
4. U.S. Patent 4,829,000

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.

Related Products:

| Catalog# | Description | Size |
|-------------|--|--------|
| 3434-005-02 | Cultrex [®] Stem Cell Qualified BME, Growth Factor Reduced PathClear [®] | 5 ml |
| 3400-010-03 | Cultrex [®] Stem Cell Qualified Laminin I, PathClear [®] | 1 mg |
| 3420-001-03 | Cultrex [®] Stem Cell Qualified Human Fibronectin, PathClear [®] | 1 mg |
| 3421-001-03 | Cultrex [®] Stem Cell Qualified Human Vitronectin, PathClear [®] | 200 µg |

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