Cultrex® Stem Cell Qualified Reduced Growth Factor Basement Membrane Extract, PathClear®

Catalog Numbers: 3434-001-02 (1.0 mL)
3434-005-02 (5.0 mL)
3434-010-02 (2 x 5.0 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 Stem Cell Qualified Reduced Growth Factor Basement Membrane Extract (RGF BME) has been shown to provide an effective feeder-free surface for the attachment and maintenance of undifferentiated human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). It is useful for promoting the expansion of pluripotent stem cells or for the study of stem cell differentiation.

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:

- Promotes the attachment of human iPSCs.
- Effectively maintains human iPSCs in a pluripotent state in a feeder-free culture.

COATING PROCEDURES:

Thaw Cultrex Stem Cell Qualified RGF BME 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 Stem Cell Qualified RGF BME, which require different thicknesses and concentrations. Some applications, such as propagation of hESCs and iPSCs in feeder-free culture, require a thin layer coating and not a thick gel; therefore, the thin layer method should be used.

Thin Layer Method for Stem Cell Propagation in Feeder-free Culture (non-gelling):

1. Thaw Cultrex BME as stated above.
2. Mix Cultrex BME by slowly pipetting solution up and down; be careful not to introduce air bubbles.
3. Dilute Cultrex BME to desired concentration in **cold** serum-free medium. Empirical determination of the optimal coating dilution for your application may be required. A 1:100 dilution is recommended for the propagation of stem cells.
4. Add a sufficient amount of solution to cover the entire area onto growth surface. 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.**
   Note: *The coated plates can be prepared in advance. Follow the procedures below:*
7. Follow Steps 1 to 4; then seal the plates with Parafilm® and store for up to two weeks in a refrigerator at 2-8 °C.
8. Prior to use, incubate coated plates at room temperature for one hour.
9. Continue with Step 6.

Thick Gel Method:

1. Thaw Cultrex BME as stated above.
2. Mix Cultrex BME by slowly pipetting solution up and down; be careful not to introduce air bubbles.
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.

DATA EXAMPLES:

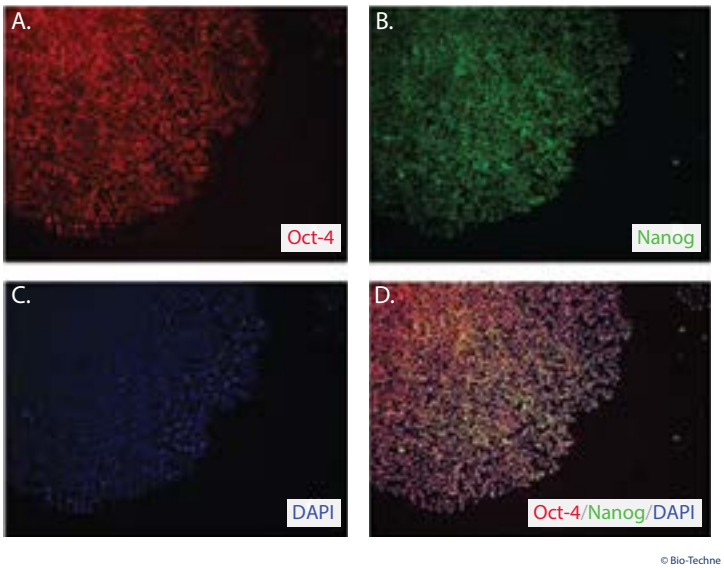

Figure 1: Immunostaining of H9 hESCs Cultured on Cultrex Stem Cell Qualified RGF BME. H9 human embryonic stem cells after four passages on Cultrex® Stem Cell Qualified RGF BME, maintain expression of pluripotency markers Oct-4 **(A)** and Nanog **(B)**. Nuclear staining by DAPI shown on panel **(C)** and merged image of Oct-4, Nanog and DAPI shown on panel **(D)**. *Images courtesy of the Yanik lab, MIT.*

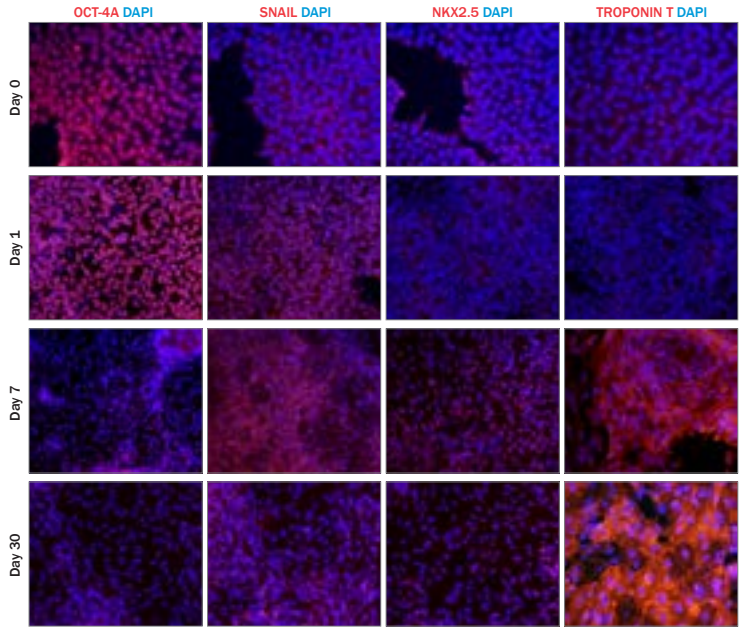

Figure 2: iPSC-derived Cardiomyocytes Differentiated on Cultrex Stem Cell Qualified RGF BME Express Stage-specific Markers. JOY6 human iPSCs were differentiated with the StemXVivo® Cardiomyocyte Differentiation Kit (R&D Systems, Catalog # SC032B), which features Cultrex Stem Cell Qualified RGF BME (Catalog # 3434-001-02) as a component, and assessed at select time points for stage-specific marker expression. The pluripotency marker Oct-4A is highly expressed during early differentiation (Day 0) and is subsequently downregulated. Expression of the mesoderm marker, Snail, is expressed intermediately during differentiation (Day 1). The cardiomyocyte markers NKX2.5 and Troponin T are not present in cells during early (Day 0) and intermediate (Day 1) differentiation and become more highly expressed during later stages of differentiation (Day 7, Day 30).

REFERENCES

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