Cultrex™ ReadyBME

Stem Cell Qualified, Reduced Growth Factor

Catalog Number: 3434-050-RTU (50 mL)

PRODUCT DESCRIPTION

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. These proteins derived from the extracellular matrix are frequently used to support the attachment, maintenance and expansion of stem cells, including human embryonic (ESCs) and induced pluripotent stem cells (iPSC) culture under feeder free conditions.

Cultrex ReadyBME is a prediluted and ready-to-use version of Cultrex Stem Cell Qualified Reduced Growth Factor BME (R&D Systems®, Catalog # 3434-005-01). As a ready-to-use coating solution, Cultrex ReadyBME eliminates the need for careful thawing and handling required when using standard Cultrex BME formulations. It increases workflow efficiency and user-to-user consistency by being provided in a form that can be taken directly from the refrigerator and immediately used to coat cell culture surfaces.

INTENDED USE

Cultrex Ready BME has been shown to be an effective feeder-free surface for the attachment and maintenance of undifferentiated human embryonic stem cells and induced pluripotent stem cells. It is useful for promoting the expansion of pluripotent stem cells or for the study of stem cell differentiation.

PRODUCT SPECIFICATIONS

Concentration	80-120 µg/mL
Source	Murine Engelbreth-Holm-Swarm (EHS) tumor
Storage Buffer	Dulbecco's Modified Phosphate Buffer
Stability	Product is stable for 12 months from date of manufacture. See lot specific Certificate of Analysis for expiration date.
Storage	Store at 2-8 °C.

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.

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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 negative for the presence of bacteria and fungi.
- Endotoxin concentration ≤ 1 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:

Work rapidly and on ice to keep the product at 2-8 °C. Cultrex™ ReadyBME is provided at the optimized concentration for stem cell propagation; we don't recommend further diluting Cultrex ReadyBME.

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

1. Working under sterile conditions, pipette enough Cultrex ReadyBME to cover the entire growth area of a cell culture plate.

Dishes/Plates	Growth area (cm^2)	Suggested Coating Volume (µL)
T-75	75	8000
T-25	25	3000
100 mm	55	6000
60 mm	21	2000
35 mm	9	1000
6 well	9.5	1000
12 well	3.8	500
24 well	1.9	250
48 well	0.95	100
96 well	0.32	50

2. Incubate coated object at room temperature for one hour or at 37 °C for 30-45 minutes.
   Note: *Longer coating incubation times may be required for some stem cell lines. We recommend either coating the object at 37 °C for up to 3 hours or overnight at 2-8 °C.*
3. After polymerization, aspirate the 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:*
4. If storing the coated plates for future use, add phosphate buffered saline (PBS) after aspirating the coating solution. Then seal the plates with Parafilm® and store for up to one week in a refrigerator at 2-8 °C.
5. Prior to use, incubate coated plates at room temperature for one hour, aspirate the PBS and then plate cells.

DATA EXAMPLES:

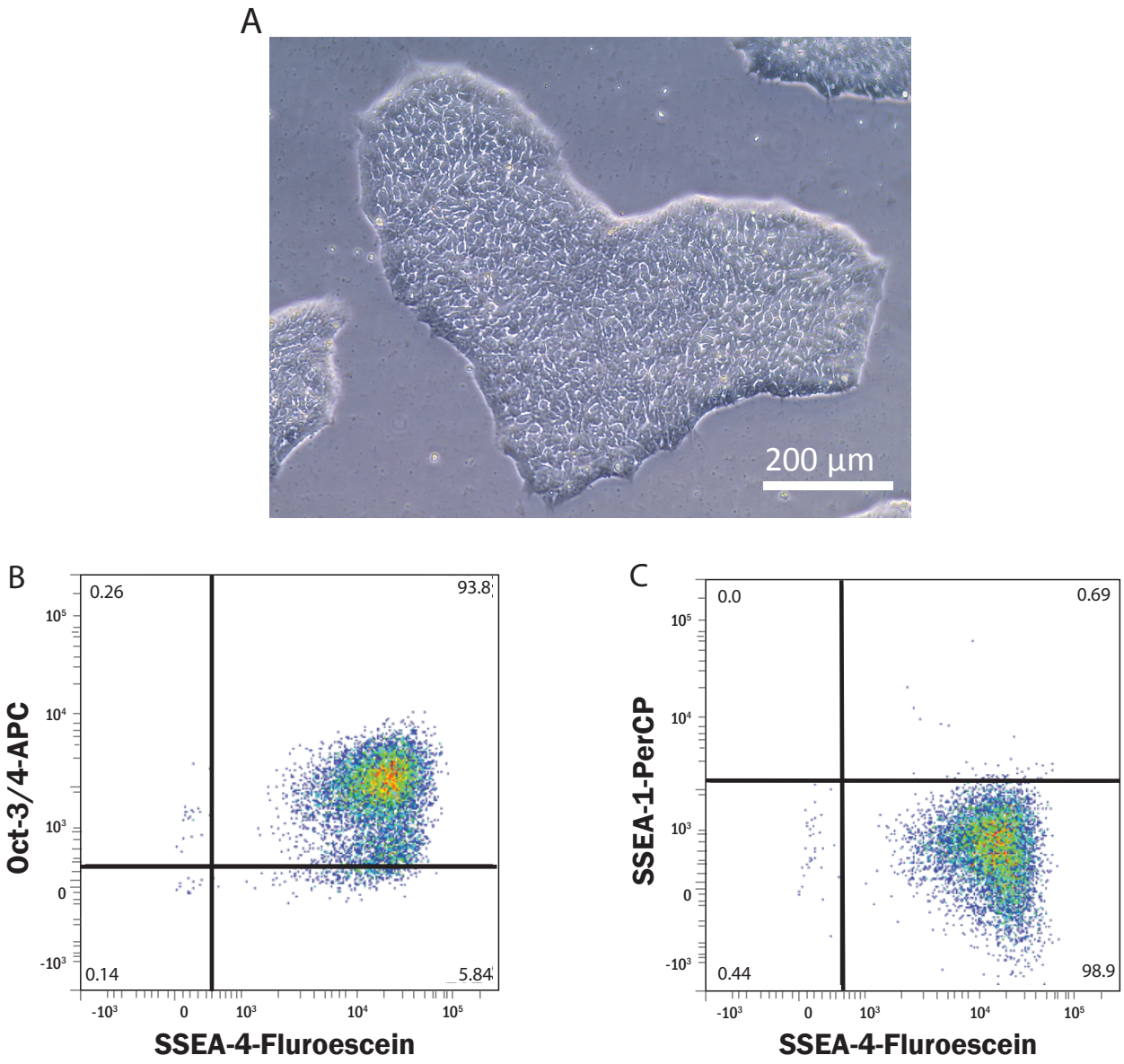

Figure 1: Maintenance of Human Induced Pluripotent Stem Cells. JOY6 human induced pluripotent stem cell (hiPSC) colonies were successfully cultured for > 10 passages using Cultrex™ ReadyBME. **A)** Representative image of JOY6 hiPSC colonies cultured on Cultrex ReadyBME. **B)** hiPSCs cultured on Cultrex ReadyBME retain stemness markers as shown by flow cytometry using a Human/Mouse Oct-3/4 APC-conjugated Antibody (R&D Systems®, Catalog # IC1759A) and Human/Mouse SSEA-4 Fluorescein-conjugated Antibody (R&D Systems, Catalog # FAB1435F). **C)** hiPSCs remain negative for SSEA-1 expression as detected using a Human/Mouse SSEA-1 PerCP-conjugated Antibody (R&D Systems, Catalog # FAB2155C).

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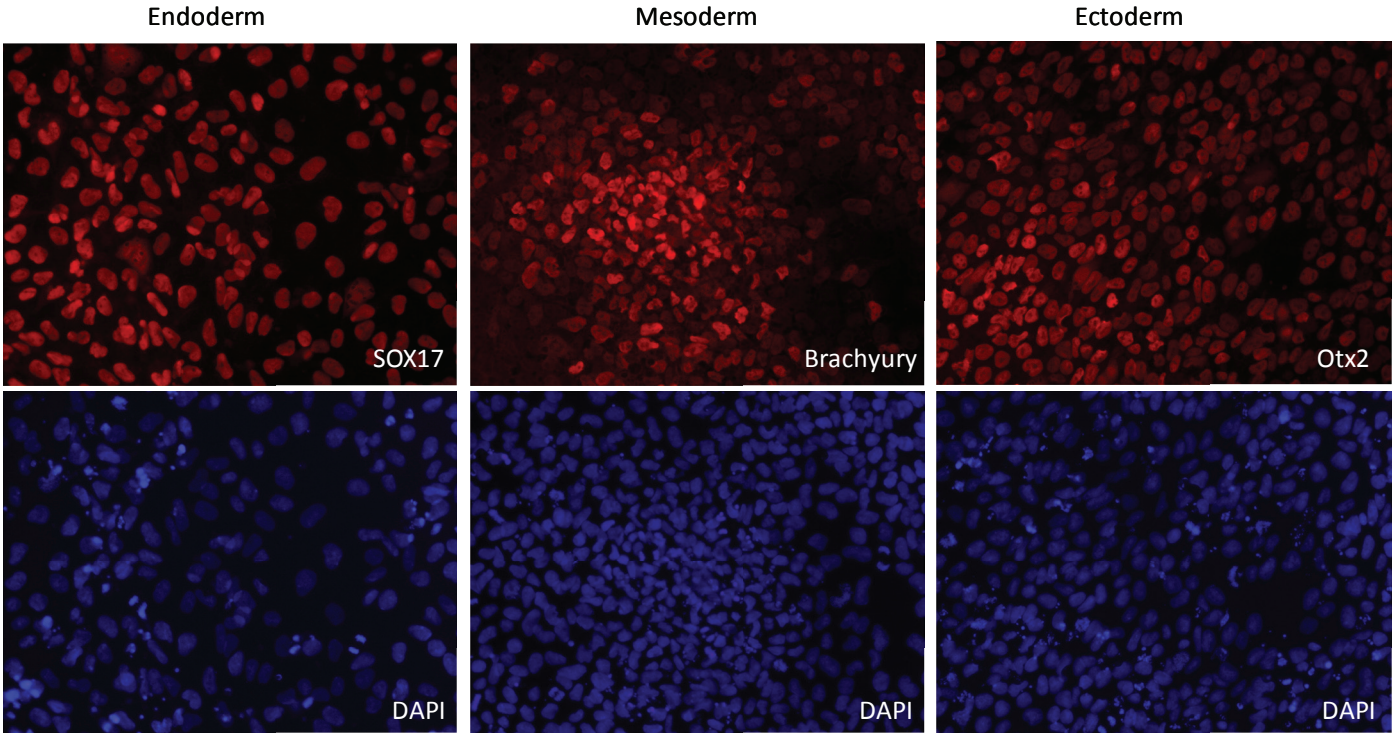

Figure 2: Differentiation of Human Induced Pluripotent Stem Cells. IBJ6 human induced pluripotent stem cells were cultured for 6 passages using Cultrex™ ReadyBME. Pluripotency was tested using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems®, Catalog # SC027B), which differentiates hiPSCs into the three germ layers. Immunocytochemistry showed that IBJ6 hiPSC successfully differentiated into endoderm (SOX17), mesoderm (Brachyury), and ectoderm (Otx2). Cells were counterstained with DAPI (R&D Systems, Catalog # 5748).