Stem Cell Qualified ECM Gel

Stem Cell ECM Substrate

Cat. # CC131-5ML

pack size: 5 mL

Store at -20ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

Stem Cell Qualified ECM Gel is a soluble basement membrane extract (BME) purified from Engelbreth-Holm-Swarm (EHS) tumor that has been pre-qualified for human ES/iPS cell culture. The matrix polymerizes at 20-40°C to form a reconstituted basement membrane. ECM Gel contains laminin, collagen IV, entactin and heparan sulfate among other ECMs. The matrix has been extensively tested and optimized for human ES/iPS cell culture.

- Eliminates the need for time-consuming prescreening and lot identification.
- Provides an optimized ECM coating necessary for long term culture of pluripotent human ES/iPS cells.
- Supports feeder-free culture of human ES/iPS cells in serum-free media.
- Compatible with all feeder free human ES/iPS cell expansion media (i.e. mTeSR®, PluriSTEM™)

Storage

Store at -20ºC. Thaw overnight on ice in a 2-8°C refrigerator. Minimize freeze thaws by aliquoting into one-time use aliquots. Store aliquots in either a -20°C or -80°C freezer until ready for use. Do not store in a frost-free freezer.

Note: *Stem Cell Qualified ECM Gel is extremely temperature sensitive and may polymerize at room temperature or higher temperature within 5-10 minutes. Keep on ice at all times and use prechilled pipette tips, tubes and plates.*

Quality Control

Appearance: Red, clear of particulates

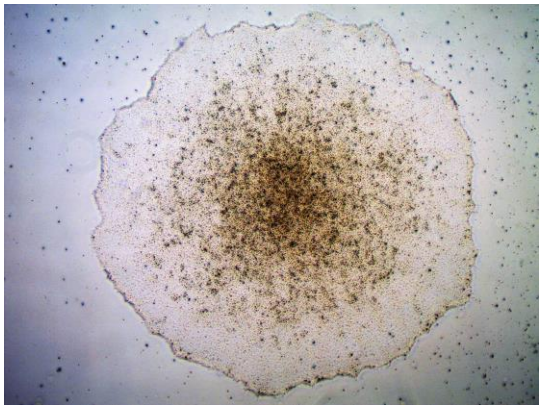
Endotoxin Level: < 20 EU/mL

Microbial Contamination: Pass

Mycoplasma: Negative

Functional Assay: <5% spontaneous differentiation for 3 passages using Human iPS cells

A



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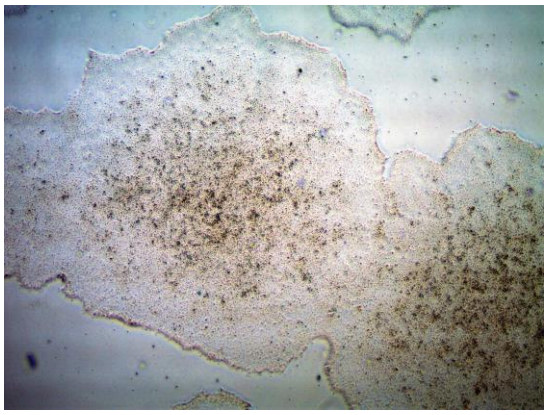

Figure 1. Growth of PBMC-derived human iPS cells grown in serum-free media on Stem Cell Qualified ECM Gel (A) or Matrigel® (B) after 5 passages. Pluripotent morphology of iPSCs grown on ECM Gel is identical to Matrigel® substrates.

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Stem Cell Qualified ECM Gel Coating Protocol

NOTE: *Expansion of human pluripotent stem cells requires culture ware that is coated with prequalified ECM proteins. Below are general guidelines for the coating of 6-well plates using the Stem Cell Qualified ECM Gel (CC131). All procedures should be performed under aseptic conditions in a biological safety cabinet.*

1. Thaw the Stem Cell Qualified ECM Gel in the 2-8°C fridge one day before use. Once thawed, maintain ECM Gel on ice at all times and use pre-cooled medium and pipettes to avoid gelling of the product.

   IMPORTANT: *Do not thaw ECM Gel at temperatures higher than 15ºC to avoid gelling.*

2. Dilute the ECM Gel 1:80 with cold DMEM/F12 (D6421) medium. Scale up or down according to the volumes required. Below is an example for coating a 6-well plate with 1.5 mL of 1:80 diluted ECM Gel.

   - To make a 1:20 dilution, add 0.5 mL ECM Gel to 9.5 mL cold DMEM/F12 (D6421) or DMEM (D5796) medium into a 15 mL conical tube. Total volume = 10 mL.
   - A further 4-fold dilution is required to make the final 1:80 dilution. Add 2.5 mL of 1:20 diluted ECM Gel to 7.5 mL cold DMEM/F12 medium. Total volume = 10 mL

   NOTE: *The recommended dilution is 1:80, however more concentrated Stem Cell Qualified ECM Gel may be used if desired.*

3. Add 1.5 mL of the 1:80 diluted Stem Cell Qualified ECM Gel to each well of a 6-well plate. Swirl the culture plates to spread the ECM Gel evenly across the surface of the plate. Store in a 2 – 8°C fridge overnight or at least 2 hours in the fridge before use. If not used immediately, parafilm wrap the ECM coated culture plates and store at 2-8˚C until ready to use. Use the ECM coated culture plates within 3-4 days.

4. Prior to seeding the cells, bring the plate back to room temperature for 10-15 minutes, remove the coating solution and add 3 mL/well of human ES/iPSC growth media. Cells can now be plated onto the newly coated plates.

   IMPORTANT: *Do not allow the plates to dry out.*

Frequently Asked Questions (FAQs):

1. **I have leftover Stem Cell Qualified ECM Gel (1:20 or 1:80 dilution). How long can I keep it?**
   Diluted ECM Gel can be kept in the fridge for 3-4 days. Within this time frame, you can either use directly for coating or further dilute to make up the 1:80 dilution. Avoid multiple freeze thaw cycles.

2. **Can precoated plates be frozen for future use?**
   No, we do not recommend freezing pre-coated plates. Pre-coated plates should be sealed with Parafilm and may be stored at 2-8°C for up to 3-4 days before use.

3. **Can I use a dilution factor higher than 1:80?**
   We recommend a maximum dilution of 1:80. Further optimization may be required for higher dilutions.

4. **My Stem Cell Qualified ECM Gel has already polymerized? How do I make it liquid again?**
   Once polymerized, ECM Gel cannot revert to a liquid form again. It is recommended to start with a fresh frozen aliquot and coat the plates again. To prevent polymerization, store ECM Gel on ice during handling.

5. **Do I need to filter the Stem Cell Qualified ECM Gel before use?**
   No, ECM Gel is cell culture tested and ready-to-use once diluted.