Product Data Sheet (DS)

Mogengel Matrix IPSC Level (Acro Certified)

PRODUCT INFORMATION

Product Description:	Mogengel Matrix IPSC Level is a soluble form of basement membrane that is purified from gene-edited mouse tumor cells grown in LDEV-free mouse populations. Reconstitution into the original basement membrane form occurs at 37°C and is mainly comprised of laminin, collagen IV, entactin, and heparin sulfate proteoglycan.
Catalog No.:	AC-M082777
Unit Sizes:	10 mL / 5 mL / 1 mL

PRODUCT SPECIFICATIONS

Source:	Gene-edited mouse tumor cells.
Storage Buffer:	Dulbecco's Modified Eagle's Medium (DMEM) with phenol red and 50 µg/mL gentamicin.
Appearance:	Mogengel Matrix IPSC Level at 4°C should have a semi-transparent, opaque consistency. A pink to yellow-pink hue should be observed.
Stability:	Product is stable for two years from date of manufacturing. Refer to the lot-specific Certificate of Analysis for expiration date.
Storage Conditions:	Store at ≤ -20°C. Avoid multiple freeze-thaw cycles. Do **NOT** store in a frost-free freezer. Product can be thawed and separated into working aliquots. **KEEP FROZEN.**

INTENDED USE

Mogengel IPSC Level is intended to be used as a supplement to specified media to culture stem cells including human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSCs). Mogengel Matrix IPSC Level provides the reproducibility and consistency required for trophoblast culture of hESCs and iPSCs and can be used for in vivo differentiation studies such as teratoma formations.

Precautions: Protective clothing should always be worn during use and safe laboratory practices should be followed when handling biohazardous materials such as human cells.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR CLINICAL PROCEDURES.

PRODUCT BACKGROUND

Basement membranes are comprised of continuous sheets of a specialized extracellular matrix. It acts as an interface between various types of cells, including muscle, neuronal, epithelial, or endothelial cells, along with adjacent stroma. Basement membranes are an essential part in the organization of tissues forming the scaffolding and support for cellular growth and cell layers. They also affect a variety of cellular mechanisms such as adhesion, migration, proliferation, and differentiation. Basement membranes are selectively degraded and regenerated during development and wound healing, forming the base scaffold for tissue reconstruction. Basement membranes also act as a major barrier to invasion by metastatic tumor cells.

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PRODUCT QUALIFICATIONS

Protein Concentration	Within 8 to 13 mg/mL, tested by BCA assay (mg/mL).
Endotoxin Level	＜ 4.5 EU/mL, tested by LAL assay (EU/mL).
Gel Formation Dilution Ratio	Diluted Mogengel Matrix with medium forms a gel within 30 minutes at 37°C and maintains it form in 37°C medium for 5 days. **Evaluated Mogengel Matrix: Medium Ratio = 1:2.*
Gel Stability	Gel forms within 30 minutes in 37°C medium and maintains form for at least 14 days. **Evaluated Mogengel Matrix: Medium Ratio = 70% (v/v).*
Stem Cell Culture	Culturing of stem cells can be performed for 7 days without differentiation in Mogengel Matrix IPSC Level coated culture plates.
Stem Cell Passage	Cultured stem cells can be passaged in Mogengel Matrix IPSC Level coated culture plates.
***In Vitro* Angiogenesis Assay**	Vascular structures are observed when HUVEC are cultured on Mogengel Matrix. **Evaluated Mogengel Matrix: Medium Ratio = 1:0 (original formulation), 2:1 (v/v).*
Sterility	No growth observed after 14 days. Testing for the detection of bacteria and fungi through cell culture. **Bacterial contaminants tested includes salmonella, murine Corynebacterium, Pasteurella pneumotropica, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, etc.*
Mycoplasma Check	No presence of mycoplasma sequence was detected in Mogengel Matrix by PCR.
Mouse Microbial Check	According to GB 14922.2-2011, the following viruses, pathogenic bacteria, parasites and bacteria in the mouse population were negative: *MHV, Ect., PVM, Reo-3, SV, MVM, PVM, Tyzzer's organism,Toxoplasma gondii； Ectoparasites, Flagellates,Ciliates,Helminths； Salmonella spp., Corynebacterium kutscheri,Pasteurella pneumotropica,Klebsiella pneumoniae, Staphylococcus aureus,Pseudomonas aeruginosa*
LDEV/LDHV Check	No presence of LDEV or LDHV sequence was detected in Mogengel Matrix by PCR

PRE-EXPERIMENT RECOMMENDATIONS

- Mogengel Matrix products are stable when stored at ≤ -20°C. Minimize freeze-thaw cycles of the product by separating into working aliquots and storing them until ready for use. Do NOT store in a frost-free freezer.
- Thaw Mogengel Matrix by first submerging the vial or working aliquot in crushed ice overnight at 4°C. For Mogengel Matrix HC (High Concentration), more time may be necessary. Please also note that Mogengel Matrix may become rehydrated after being placed on ice at 4°C after 24 to 48 hours.
- Mogengel Matrix will start to solidify into a gel at temperatures above 10°C. Remember to pre-chill all reagents

and materials before use with this product. Keep Mogengel Matrix on ice throughout the experiment to prevent unwanted gel formation.

- Make sure to always keep Mogengel Matrix on ice. Before use, use a pre-cooled pipette to gently aspirate and mix Mogengel Matrix to ensure homogeneity.

SAMPLE PROCEDURES: IPSC CULTURE

1. Equipment, reagents, consumables needed

1.1. Reagents: Mogengel Matrix IPSC Level, ROCK inhibitor, DMEM F12; mTeSR1/E8 medium; Accutase Dissociating solution, PBS(D-PBS).

1.2. Consumables: Sterile pipette tips; 6-well plates; Sterile EP tube and other consumables.

2. Preparation Protocol

2.1. Pre-experimental preparation

2.1.1. Place Mogengel Matrix into an ice box before placing it into a refrigerator held at 4°C to thaw overnight. *Do **not** allow the product to reach a temperature over 4°C when using. Always keep product on ice and dilute using cold solution or cell suspension.

2.1.2. Consumables or reagents that come into contact with Mogengel Matrix, such as sterile centrifuge tubes, pipette tips, and DMEM F12, should be chilled to 4°C before use.

2.2. Mogengel plate coating and dilution

**Tip: Mogengel Matrix dilution ratios of 1:80 to 1:100 are acceptable, which is equivalent to a concentration of 0.1 mg/mL. For iPSC culturing, we recommend coating the culturing surface with 0.013 mg/cm^2.*

2.2.1. Transfer an appropriate working amount of DMEM F12 into the chilled EP tube. All reagents should be on ice or held at 4°C.

2.2.2. Use a pipette with chilled tips to transfer 1 mL of DMEM F12 into a working aliquot of Mogengel before mixing well and transferring into another EP tube kept on ice. Repeat this step several times until the complete working amount of Mogengel has been placed into the EP tube.

*Note: Mogengel at 4°C will gradually polymerize into a gel. Please control the working temperature and time.

2.2.3. Add 1 mL per well of the Mogengel mixture and gently shake to ensure that matrix is spread across the well.

2.2.4. Transfer the plate into a 37°C incubator for incubation overnight. The minimal amount of time for use is 1 to 2 hours, but overnight is recommended for ideal cell culturing conditions.
*Note: Coated plates can be stored at 4°C and should be used within 1 week of coating. Any remaining coating solution should be aspirated before use.

***2.3.* Working medium with ROCK inhibitor**

2.3.1. Use sterile PBS to dissolve T-27632 and configure into a 10mM solution with a working concentration of 10 µM.

2.3.2. Add the working 10mM solution with 10 µM of ROCK inhibitor to the mTeSR1/E8 medium until final concentration is 10 µM.

***3.* Cell Culture - iPSC**

3.1. Thawing of iPSCs

3.1.1. Remove iPSCs from storage in liquid nitrogen or dry ice and thaw in 37°C water.

3.1.2. Disinfect frozen tube with 75% alcohol and transfer it onto the benchtop.

3.1.3. Transfer cell solution into a new 15 mL EP tube and flush the primary tube with DMEM F12 or DMEM.

3.1.4. Centrifuge the 15 mL EP tube at room temperature at 300g for 5 minutes. iPSCs are tolerant to 200 to 300g speed. Using 300g is recommended to maximize cell capture, while 200g is recommended as a standard protocol.

3.1.5. Discard the supernatant and gently resuspend iPSCs with 2 mL of ROCK inhibitor-containing working medium, and transfer it to a coated 6 well plate. Shake plate to evenly distribute cells for a final cell density of 1×10^6 cells per well.

3.1.6. Place the 6-well plate back into 37°C incubator. This should be done immediately after transfer to prevent aggregation in the center of the wells.

3.1.7. ROCK inhibitor-containing medium should be removed after 24h and continue to be cultured in non-inhibitor-containing medium.

**Note: Use of antibiotics in cell culture is not recommended as it interferes with iPSCs and their differentiation potential. The culturing environment should also be isolated from other cells, with the mycoplasma detection assays performed after two passages. If the cryopreservation solution contains DMSO, the cell thawing procedure should completed as quickly as possible due to its toxicity to cells at room temperature.*

3.2. iPSC passaging protocol

3.2.1. Discard the culture supernatant and rinse with 1 mL PBS and add 1 mL Accutase.

3.2.2. Transfer plate to a 37°C incubator for 3 minutes or keep under microscopic observation until cells are not bound to the plate. If cells remain attached, place the culture plate on your hand and gently tap on its side to release the cells from the walls.

3.2.3. Prepare a Mogengel coated 6-well plate before passaging your cells.

3.2.4. When all materials and reagents are prepared, tilt the culture plate with iPSC cells and transfer the excess Accutase solution across the surface of the cell culture plate twice to separate clumps. Transfer the remaining solution into a centrifuge tube.

3.2.5. Rinse the surface of the cell culture plate with DMEM F12 and merge with the cell solution in the centrifuge tube. Washing with DMEM F12 or PBS (at least 5% medium) is recommended for subsequent granulation and attachment.

3.2.6. Centrifuge the centrifuge tube at 300g at room temperature for 5 min.

3.2.7. Discard the supernatant and resuspend cells with ROCK inhibitor-containing medium.

3.2.8. Transfer the cells to the prepared Mogengel-coated 6-well plate. Shake the plate gently to evenly distribute cells.

3.2.9. Place the 6-well plate into a 37°C incubator for incubation.

*Note: iPSCs will rapidly differentiate and die when they grow into a single layer. In order to maintain growth and pluripotency, make sure to passage your cells before 100% cell confluency.

3.3. Cryopreservation of iPSCs

3.3.1. Prepare Accutase dissociation solution for use.

3.3.2. Follow the iPSC passaging protocol for cell isolation. Use a cell counter to ensure cell density before cryopreservation.

3.3.3. Each tube of cells should be frozen at a density of 1×10^6. Follow the centrifugation, extraction of cell medium steps in the iPSC passaging protocol. Resuspend isolated iPSC pellet in the appropriate volume of cryopreservation solution.

3.3.4. Add 1mL of the resuspended cell cryoprecipitate into a 1.5 mL freezing tube and undergo programmed cooling before transfer into liquid nitrogen for long-term storage.

COATING PROTOCOL

Mogengel Matrix can be used in several methods. Forming different gels with different thicknesses, concentrations, and consistencies can produce better results depending on the application. For example, a thin non-gel layer is more suitable

for propagating primary cells. For three-dimensional cell culturing or where structure formation is needed, a thick/thin layer gel coating method is recommended, where cells can grow on the complex layers of protein.

Thin Layer (non-Gel) Method:

1. Thaw Mogengel Matrix following the recommended pre-experiment recommendations above.
2. Using a pre-chilled pipette, mix by aspirating and dispensing slowly until homogenous. Take care not to introduce air bubbles into the solution.
3. Dilute Mogengel Matrix to the desired concentration using **cold** serum-free medium. Based on the experimental application, empirical evidence may be needed to optimize the coating concentration.
4. Add the diluted Mogengel Matrix onto surface that is to be coated. The amount added should be sufficient to cover the entire growth surface.
5. Incubate at room temperature for 1 hour.
6. Aspirate the remaining unbound material and rinse gently using serum-free medium.
7. The growth surface (or object) is ready to use.

Thin Layer Gel Coating Method:

1. Thaw Mogengel Matrix following the recommended pre-experiment recommendations above.
2. Using a pre-chilled pipette, mix by aspirating and dispensing slowly until homogenous. Take care not to introduce air bubbles into the solution.
3. Place the growth surface (or plate) on ice and pipette 50 µL per cm^2 onto the surface.
4. Transfer the surface to 37°C for 30 minutes.
5. Growth surface (or plate) is ready to use.

Thick Layer Gel Method:

1. Thaw Mogengel Matrix following the recommended pre-experiment recommendations above.
2. Using a pre-chilled pipette, mix by aspirating and dispensing slowly until homogenous. Take care not to introduce air bubbles into the solution.
3. Place the growth surface (or plate) on ice and pipette 150 – 200 µL per cm^2 onto the surface.
4. Transfer the surface to 37°C for 30 minutes.
5. Growth surface (or plate) is ready to use. Serum-free medium can be added, and cells can be cultured on top of this gel.