Mogengel Matrix (Acro Certified)

PRODUCT INFORMATION

Product Description:	Mogengel Matrix 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-M082704
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 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 Matrix is intended to be used in 2D or 3D cultures related to cell proliferation, cell differentiation, cell invasion, angiogenesis, or organoid culture, among many other applications.

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).*
Tumor Invasion	Cells pass through basement membrane matrix and into the lower compartment, tested by Transwell invasion assay. Tumor cells can be cultured on the basement membrane matrix. **Evaluated Mogengel Matrix: Medium Ratio = 1:4, 1:6, 1:8 (v/v).*
***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.

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SAMPLE PROCEDURES: ANGIOGENESIS ASSAY

1. Equipment, reagents, consumables needed

1.1. Equipment: Pipettes, carbon dioxide (CO_2) incubator, inverted microscope, low-speed centrifuge, biosafety cabinets.

1.2. Reagents: Mogengel Matrix, Dulbecco's Modified Eagle Medium (DMEM), extracellular matrix (ECM) complete medium, trypsin solution, phosphate buffer solution (PBS).

1.3. Consumables: Sterile pipette tips; 96-well cell culture plates; Sterile EP tube and other consumables.

2. Experimental methodology

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, should be chilled to 4°C before use.

2.2. Plate coating procedure

2.2.1. Prepare the EP tube by placing it on ice. Add each component according to the following table and based on selected ratios (Mogengel: DMEM) chosen.

Mogengel Ratio (Mogengel：DMEM）	Stock solution	2:1	1:1
DMEM(μL)	0	40	60
Mogengel(μL)	100	80	60

**Note: In most cases, a 2:1 ratio is most appropriate.*

2.2.2. After mixing the components in the selected ratio above, add 50μL per well in the 96-well plate and solidify in a 37°C incubator for at least 1 hour.

2.3. Incubating human umbilical vein endothelial cells (HUVECs)

2.3.1. HUVECs were selected for studies into pancreatic enzyme digestion. Cell density was first adjusted to 4×10^5cells /mL by using ECM medium.

2.3.2. Use the Mogengel-coated 96-well plate from Step 2.2.2 and add 50 μL per well of the cell suspension for a final count of 2×10^4 cells / well.

2.3.3. Incubate the 96 well plate in the CO_2 incubator at 37°C for 4h and 24 h.

2.3.4. Microscopic observations were made at 4 and 24 h for vascular structure formation.

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.

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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.

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