Mogengel Matrix (HC) (Acro Certified)

PRODUCT INFORMATION

Product Description:	Mogengel Matrix (HC) (High Concentration) 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-M082724
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 (HC) at 4°C should have a semi-transparent, opaque consistency. A pink to yellow-pink hue should be observed. Their viscosity is higher than the ordinary concentration of matrix glue.
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 (HC) is intended to be used for in vivo experiments, including patient-derived xenografts, cell-derived xenografts, as well as in vitro angiogenesis assays.

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 16 to 26 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 = 1:4.*
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).*
Subcutaneous Tumor Xenograft	Subcutaneous tumor formation with the addition of Mogengel Matrix (HC GFR) to promote proliferation of tumor cells. **Evaluated Mogengel Matrix Ratio > 50%.*
Tumor Organoid Culture	Tumor cell line-derived organoids can be cultured in suitable media with Mogengel Matrix (HC GFR) > 33%.
***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: SUBCUTANEOUS TUMOR FORMATION

1. Equipment, reagents, consumables needed

1.1. Equipment: Biosafety cabinet, cell incubator, low temperature horizontal centrifuge, inverted microscope.

1.2. Reagents: Mogengel Matrix (HC), basic medium, medium containing 10% fetal bovine serum, phosphate buffered solution (PBS), 0.25% trypsin solution.

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. Experimental Procedure

2.2.1. Select cells in good condition with logarithmic growth. Discard the supernatant before adding 2 mL of PBS solution to wash gently. Discard liquid after use.

2.2.2. Add 1 mL of 0.25% trypsin solution for cell digestion into the cell culturing plate. Let it sit for 10 seconds before discarding the excess trypsin solution. Continue digestion at room temperature for 1 to 3 minutes with residual trypsin.

2.2.3. When cells detach from the cell plate and become round*, add 1 mL medium containing 10% fetal bovine serum to terminate digestion. Carefully blow the cells apart and collect the cell suspension in a 15 mL plastic centrifuge tube.
**Note: Cell edge must first be cleared before being digested with trypsin.*

2.2.4. Centrifuge the cell suspension at 1000 rpm for 3 minutes and discard the supernatant. After washing the cells with PBS, resuspend cells in a serum-free basal medium. Aliquot 10 µL for cell counting.

2.2.5. For different cell types, follow the following steps for vaccinations:

- ***HepG2 cells:*** Each mouse is vaccinated with 5 million cells. Based on the calculated cell density, the relevant volume of cell suspension is first placed into a separate, sterile 1.5 mL plastic centrifuge tube and centrifuged at 1000 rpm for 3 minutes. Discard the supernatant and add serum-free medium or PBS until the total volume is 300 µL.
- ***HCT - 116 cells:*** Each mouse is vaccinated with 1 million cells. Based on the calculated cell density, the relevant volume of cell suspension is first placed into a separate, sterile 1.5 mL plastic centrifuge tube and centrifuged at 1000 rpm for 3 minutes. Discard the supernatant and add serum-free medium or PBS until the total volume is 300 µL.
- ***MIA - PaC - 2 cells:*** Each mouse is vaccinated with 10 million cells. Based on the calculated cell density, the relevant volume of cell suspension is first placed into a separate, sterile 1.5 mL plastic centrifuge tube and centrifuged at 1000 rpm for 3 minutes. Discard the supernatant and add serum-free medium or PBS until the total volume is 500 µL.

2.2.6. Mix in Mogengel Matrix with the cell suspension at a 1:1 ratio at 4℃, and keep on ice until use.

2.3. Subcutaneous injection of mice

2.3.1. Nude mice are injected subcutaneously into the right back with the Mogengel-cell suspension solution. During inoculation, the needle should be inserted deeper than subcutaneous tissue, around 1 cm deep, to reduce the overflow of solution from the injection site. Injection volume should be 100 µL. For MIA-PaC-2 cells, inject 200 µL.

2.3.2. After injection, mice are placed back into the cage for further feeding while measuring tumor volume regularly according to experimental requirements.

2.3.3. Mice are euthanized before tumor volume reaches 2000 mm^3. Tumor is then removed and photographed for comparison.

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.