Mogengel Matrix (GFR) (Acro Certified)

PRODUCT INFORMATION

Product Description:	Mogengel Matrix (GFR) (Growth Factor Reduced) 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-M082701
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 (GFR) 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 (GFR) is intended to be used in organoid/3-D cell culturing and applications that require extracellular matrix scaffolding. This includes applications including maintaining cellular growth and differentiation of various cell types, including stem, endothelial, epithelial, and others. It is also intended for applications regarding a more demanding preparation of basement membrane such as tubular osteocyte signaling studies or as a base for mouse mammalian epithelial cell expression studies (basement membrane matrices can reduce the background signals triggered by external addition of growth factors).

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).*
Organoid Culture	Mouse intestinal, liver ductal, and airway organoids can be cultured in suitable media with Mogengel Matrix GFR > 70%. **Evaluated Mogengel Matrix: Medium Ratio > 70% (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.

SAMPLE PROCEDURES: MOUSE INTESTINAL ORGANOID CULTURE

1. Equipment, reagents, consumables needed

1.1. **Equipment:** Biosafety cabinet, pipettes, carbon dioxide (CO_2) incubator, inverted microscope, centrifuge (low-speed).

1.2. ***Reagents:*** Mogengel Matrix GFR, Mouse Intestinal Organoid Kit, anti-adherence rinsing solution, DPBS, DPBS containing 0.1% bovine serum albumin (BSA), penicillin-streptomycin solution, 0.5M EDTA solution (pH = 8.0).

1.3. ***Consumables:*** Sterile pipette tips, cell culture plates (48-well plate), cell culture dishes (diameters: 3.5,6,10 cm), sterile forceps, sterile tissue scissors, 70 μm strainer, surgical knife, 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.

2.1.3. Prepare Mouse Intestinal Organoid complete medium as directed.

2.1.4. Prepare plenty of DPBS containing 1% BSA.

2.2. **Experimental Operating Procedures**

2.2.1. Sacrifice the mouse ethically following the pre-established animal ethics guidelines and operating conventions. If regulatory approval, e.g. IRB, is required, please obtain one before proceeding.

2.2.2. Prepare a 6 cm dish and add ice-cold DPBS containing 0.1% BSA for later use. Keep on ice.

2.2.3. Under sterile conditions, remove 3 to 5cm of intestinal tissue near the gastric end and place onto the chilled DPBS dish prepared in Step 2.2.2.

2.2.4. Cut the intestinal cavity lengthwise and scrape off the surface villi.

2.2.5. Wash twice before cutting into a 2 mm wide intestinal segment.

2.2.6. Wash twice again, and then transfer onto the pre-cooled DPBS containing 5 mM EDTA and wait 20 min for digestion. Keep on ice.

2.2.7. After digestion, transfer the tissue fragments onto a new dish containing DPBS to wash, and repeat twice to remove traces of EDTA.

2.2.8. Rinse the 5 mL pipette tip using anti-adherence rinsing solution. Resuspend the intestinal fragments with DPBS containing 0.1% BSA by aspirating/dispensing the mixture 3 to 4 times.

2.2.9. Collect the resulting suspension and filter it with the 70 μm strainer. Repeat this step twice.

2.2.10. Centrifuge the remaining suspension for 3 min at 300g and remove the supernatant. Resuspend using 1 mL of DPBS containing 0.1% of BSA. Aliquot 20 μL of suspension for microscopic examination and counting.

2.2.11. After counting, aspirate the suspension containing the required amount of crypts and centrifuge at 300g for 3 minutes.

Remove the supernatant and resuspend using Mogengel matrix. The volume of Mogengel recommended for use depends on the size of the resulting pellet. Approximately 70 to 100 crypts should be plated in 10 µL of Mogengel

*CAUTION: Do not overly dilute Mogengel (the ratio should be over 70%). Ratios below this may inhibit the proper formation of solid droplets.

2.2.12. Plate Mogengel-containing suspension at the bottom of a 48-well cell culture plates in droplets of 12 to 20 µL around the center of the well. Plate as quickly as possible to prevent Mogengel gelling within the tube or pipette tip. Do NOT let Mogengel touch the well walls. Please refer to the following for the droplet volumes for different kinds of well plates.

Number of Wells	96	48	24
Volume of Crypt: Mogengel Solution (µL)	3 to 8	12 to 20	20 to 30

2.2.13. Place the culture plate into a 37°C carbon dioxide incubator for 15 minutes for Mogengel to solidify.

2.2.14. Prepare complete medium of mouse intestinal organoids and add to the 48 well plate at 250µL per well. Please add the mouse intestinal organoids medium slowly to avoid damaging the solidified structure.

2.2.15. Afterwards, incubate the 48-well plate in the CO_2 incubator at 37°C. *Please note that medium should be changed every 3 days to avoid damaging the Mogengel structure.

2.2.16. Closely monitor the growth status of organoids. Ideally, mouse intestinal organoids should be established within 5 to 7 days.

3. Splitting and Passaging Mouse Intestinal Organoids

3.1. Additional Reagents required:

3.1.1. Reagents: Melted Mogengel, Mouse Intestinal Organoid Kit, Organoid Dissociation Solution, Epithelial Organoid Basal medium

3.1.2. Consumables: Sterile pipette tips, cell culture plates (48-well plate), sterile EP tube and other consumables.

3.2. Experimental operating procedures

3.2.1. Keep the original culture medium, and gently scrape off the extracellular Mogengeland organoid mixture with a pipette tip and transfer to a 1.5 mL tube. Pipette the organoid suspension until the solution is homogenous by pipetting against the bottom of the tube to create pressure and aid the removal of Mogengel.

3.2.2. Centrifuge at 300g at 4°C for 3 min. Discard the supernatant.

- For those using the organoid dissociation solution kit, resuspend the pellet in 0.2 mL of Organoid Dissociation Solution. Aspirate and dispense before incubating at 37°C until organoids fall apart. Do **not** leave the organoids to dissociate in solution for more than 7 minutes.
- Mechanical disruption can also be performed by resuspending pellet with epithelial organoid basal medium. Aspirate and dispense the suspension 5-10 times and centrifuge again at 300g at 4°C for 3 minutes. Wash and place on ice.

3.2.3. After shearing is complete, wash twice with 1 mL of Epithelial Organoid Basal medium and centrifuge at 300g for 3 min.

3.2.4. Resuspend the organoid pellet with an appropriate amount of Mogengel and place on ice after resuspension.

*CAUTION: Resuspension time should not be more than 30s to avoid premature gelling of Mogengel. Do not overly dilute Mogengel (the ratio should be over 70%, *v/v*) to ensure structural integrity.

Place the mixed suspension of Mogengel and organoid into the center of a 48-well cell culture plate and avoid the suspension from contacting the side wall of the plate, with 15 µL per well.

3.2.5. Put the inoculated culture plate into the 37°C CO_2 incubator and incubate for 15 minutes until Mogengel has solidified.

Prepare a complete medium of mouse small intestinal organoid and add onto a 48-well cell culture plate for 250 µL per well.

3.2.7. Place into a humidified incubator at 37°C incubator with a 5% (*v/v*) CO_2.

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.