For Organoid Culture, Basement Membrane Matrix

Cat #: A-MGL55

Size: 5mL, 10mL

Storage: ≤-20°C

Product Description

Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between endothelial, epithelial, muscle, or neuronal cells and their adjacent stroma. Basement membranes are degraded and regenerated during development and wound healing. They not only support cells and cell layers, but they also play an essential role in tissue organization that affects cell adhesion, migration, proliferation, and differentiation. Basement membranes provide major barriers to invasion by metastatic tumor cells.

For Organoid Culture, Basement Membrane Matrix is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumor. For Organoid Culture, Basement Membrane Matrix at 37°C to form a reconstituted basement membrane. The major components of For Organoid Culture, Basement Membrane Matrix include laminin, collagen IV, entactin, and heparin sulfate proteoglycan.

Intended Use

This is the selected Basement Membrane Matrix to prove organoid culture, if you encounter organoids that cannot be cultured with other types of substrate glue, the use of this series of Basement Membrane Matrix can ensure the effectiveness and reproducibility of the experiment. For example, mice intestinal organoids, lung organoids, human brain organoids and so on.

Product Parameter

1. Source: Mouse Tumor
2. Color: Phenol Red-containing Basement Membrane Matrix is yellow-pink, and Phenol Red-free Basement Membrane Matrix is translucent light yellow;

3. Form: Standard Basement Membrane Matrix dissolved at 4°C, a transparent liquid state; High concentration Standard Basement Membrane Matrix after 0°C solution, transparent liquid state, 4°C for a long time to show a semi-gel.
4. Concentration: Protein concentration ranges from 8 to 26 mg/mL
5. Endotoxin: ≤ 4.5 EU/mL
6. Gel time: 5-30 min gel at room temperature, the speed of gel formation is accelerated when the temperature is from 22°C to 37°C.

Material Qualifications

- Routine screening of mouse colony pathogens by mouse antibody product (MAP) tests
- Testing for bacteria, fungi and mycoplasma to ensure negative results
- Extensive PCR testing for a variety of pathogens including LDEV to ensure strict control of raw materials used in the production process
- Extraction from LDEV-free mouse tumor cells
- Gel stability testing at 37°C for 14 days
- Detection of endotoxin levels using serological methods
- Biological function verification of each lot (organoid culture and differentiation experiments; Subcutaneous tumor formation test; Stem cell culture; Angiogenesis experiment etc.)

Coating Procedures

Thaw Basement Membrane Matrix overnight at 2-8°C. Refrigerator temperatures may vary, therefore it is recommended to keep Basement Membrane Matrix on ice in a refrigerator during the thawing process. Thawed Basement Membrane Matrix solidifies quickly at temperatures above 15° C; when working with Basement Membrane Matrix, keep it on ice to prevent untimely gelling.

There are many applications for Basement Membrane Matrix which require different thicknesses and concentrations. A thick gel is needed for applications such as endothelial cell formation of capillary-like structures (Tube Formation Assay), the differentiation of rat aorta tissue into capillary-like structures (Aortic Ring Assay), epithelial organoid formation, or

tumor organoid formation. Some applications, such as propagation of primary cells, require a thin layer coating and not a thick gel; therefore, the thin layer method should be used.

Thick Gel Method:

1. Thaw Basement Membrane Matrix as stated above.

2. Mix Basement Membrane Matrix by slowly pipetting solution up and down; be careful not to introduce air bubbles.

3. Pipette 200-300 µL per cm^2 onto the growth surface.

4. Place coated object at 37° C for 30 minutes.

5. Coated objects are ready for use.

Thin Layer Method (non-gelling):

1. Thaw Basement Membrane Matrix as stated above.

2. Mix Basement Membrane Matrix by slowly pipetting solution up and down; be careful not to introduce air bubbles.

3. Dilute Basement Membrane Matrix to desired concentration in cold serum-free medium. A 1:100 dilution is recommended for the propagation of primary cells. Empirical determination of the optimal coating concentration for your application may be required.

4. Add a sufficient amount of solution to cover the entire growth surface area. A volume of 300 µL per cm^2 is recommended.

5. Incubate coated object at room temperature for one hour.

6. Aspirate coating solution and immediately plate cells. Do not allow coated surface to dry out.

Protocol for Establishment of Organoids (Mouse Intestinal Organoids is an example)

A. Establishment of Organoids from Primary Mouse Intestinal

1. Equipment, reagents and consumables

1.1 Equipment: Biosafety cabinet, pipette, carbon dioxide incubator, inverted microscope, centrifuge (Low-speed).

1.2 Reagent: Basement Membrane Matrix (Cat: A-MGL03 / A-MGL55), Mouse Intestinal Organoid Kit (Cat: MA-0817H006L), DPBS, Anti-Adherence Rinsing Solution (Cat: MB-0818L03L), Penicillin-Streptomycin Solution, DPBS containing 0.1% BSA, 0.5M EDTA Solution (pH=8.0).

1.3 Consumables: sterile pipette tips; cell culture plate (48-well for example in this protocol); Cell culture dish (diameter: 3.5 cm, 6 cm and 10 cm), sterile forceps, sterile tissue scissors, 70 μm strainer, and surgical blade, sterile EP tube and other consumables. (Or be adjusted according to the experimental design).

2. Preparation before Experiment

2.1 Put the Basement Membrane Matrix in the ice box and put it in the refrigerator at 4°C so that the Basement Membrane Matrix can slowly melt overnight; (Do not allow this product to warm up above 4°C during manipulation. Keep the product on ice and dilute using ice-cold solutions or cell suspensions.)

2.2 Prepare Mouse Intestinal Organoid Complete Medium as directed.

2.3 Prepare plenty of DPBS containing 0.1% BSA.

3. Experimental operating procedures

3.1 Sacrifice mice according to the experimental animal ethics and operating norms approved by the unit.

3.2 Prepare 6 cm dishes and add ice-cold DPBS containing 0.1% BSA for later use. (kept on ice)

3.3 Under sterile conditions, remove 3~5cm intestinal tissue near the gastric end and put into the “3.2” pre-cold DPBS.

3.4 Cut the intestinal cavity lengthwise, gently scrape off the surface villi, wash twice, cut to a 2mm wide intestinal segment, wash twice, and transfer to pre-cold DPBS containing 5mM EDTA, wait 20min for digestion (kept on ice).

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

3.6 Rinse the 5 mL pipette tip by Anti-Adherence Rinsing Solution. Resuspend intestinal fragment with DPBS containing 0.1% BSA, pipetting the mixture up and down 3~4 times, collect the suspension and filter it with a 70 μm strainer. And repeat this step once more time.

3.7 Centrifuge 300 g for 3 min to collect crypts, resuspend using 1mL of DPBS containing 0.1% BSA, take 20 μL of suspension for microscopic examination and crypt counting.

3.8 Counting is completed, aspirate the suspension containing the required amount of crypts, centrifuge 300g for 3 min.

3.9 Aspirate the supernatant and resuspend the crypts in Basement Membrane Matrix. The Basement Membrane Matrix should be kept on ice to prevent it from solidifying. Perform the process as quickly as possible. The volume of Basement Membrane Matrix used depends on the size of the pellet. Approximately 70~100 crypts should be plated in

10 µL of Basement Membrane Matrix.

CRITICAL: Do not overly dilute the Basement Membrane Matrix (Basement Membrane Matrix ratio should be >70% (Basement Membrane Matrix vol/Total vol)), as this may inhibit the proper formation of the solid droplets.

3.10 Plate the Basement Membrane Matrix containing crypts at the bottom of 48-well cell culture plates in droplets of 12 ~20 µL each around the center of the well. Proceed with plating as quickly as possible, as the Basement Membrane Matrix may solidify in the tube or pipette tip. Do not let the Basement Membrane Matrix touch the wall of wells.

The amount of crypts - Basement Membrane Matrix mixture added for different plates is shown in the table below:

Number of wells	96	48	24
Volume of crypts - Basement Membrane Matrix mixture (µL)	3~8	12~20	20~30

3.11 Place the culture plate into carbon dioxide incubator at 37°C for 15 min to let the Basement Membrane Matrix solidify.

3.12 After Basement Membrane Matrix was completely solidified, the prepared complete medium of mouse intestine organoid was added to a 48-well plate at 250 µL per well.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.