For iPSC/Stem Cell, Basement Membrane Matrix

Cat #: A-MGL77

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

Intended Use

It is a mature growth substrate for feeder-free hES culture. It's used in combination with specific media to culturize stem cells (e.g, hESC, iPSC). The screened stem cell Basement Membrane Matrix provide the reproducibility and consistency required for trophoblast culture of human embryonic stem cells and induced pluripotent stem cells, and can also be used in vivo differentiation studies, such as teratoma formation.

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.

iPSCs Experiment Procedures

1. Materials

1.1 Reagent: Basement Membrane Matrix (Cat: A-MGL77); ROCK inhibitor (Y-27632); DMEM/F12; mTeSR1/E8 medium; Accutase Dissociating solution, PBS (D-PBS).

1.2 Consumables: sterile tips, 6-well plate (or other well number of plates, this protocol will use a six-well plate as an example), sterile EP tube.

2. Preparation

2.1. Material pre-cooling

2.1.1 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.);

2.2.2 Supplies or reagents that come into contact with Basement Membrane Matrix, such as sterile centrifuge tube, sterile tips and DMEM/F12, were be pre-cooled at 4°C in advance.

2.2. Dilute the Basement Membrane Matrix (Cat: A-MGL77-5mL) at (1:80 - 1:100)

(Tip: 1:80 - 1:100 are all appropriate, equivalent to Basement Membrane Matrix concentration of 0.1mg/mL. For iPSC culture, we recommends coating at a concentration of approximately 0.013 mg/cm², for example, after diluting 12.5 mg/mL of Basement Membrane Matrix 1:100, the coating volume of each 6-well plate is 1 mL)

2.2.1. Transfer appropriate of DMEM/F12 at 4°C into the cooled EP tube;

2.2.2. Use a pipette with the pre-cooled tips to transfer the DMEM/F12 to the aliquot of Basement Membrane Matrix, mixed well and then transferred to the other EP tube (kept on ice). Repeat this step 2-3 times until the matrix gel is completely pipetted into the EP tube, replenish the dilution to a final concentration that meets the requirements of your own design after that;

2.2.3. The mixture is as a reserve for subsequent coating.

2.3. Plate coating procedure

2.3.1 Add the 1mL/well Basement Membrane Matrix mixture into the pre-cooled 6-well plate, and gently shake the plate to ensure that the mixture is evenly spread on the plate;

2.3.2 Transfer the 6-well plates to a 37°C incubator for overnight incubation (the plates can be used after incubating for 1-2 hour, but the coating for overnight incubation is better for cell culture. Coated plates with coating solution can be stored at 4°C and should be used within one week of coating. Coating solution should be aspirated just before using the plates);

2.3.3 Absorb the liquid above the coating before use.

2.4. Configure ROCK inhibitor (Y-27632) working solution

Use sterile PBS to dissolve Y-27632 and configure it into 10mM solution (1000X) with working concentration of 10uM.

2.5. Preparation of medium containing ROCK inhibitor:

ROCK inhibitor (Y-27632) with 10mM solution was added to mTeSR1/E8 medium until the final concentration was 10uM.

Note: The Basement Membrane Matrix at 4°C will gradually polymerization into glue. Please strictly control the operating temperature and the operating time.

3. Cell culture

3.1. iPSC thawing

3.1.1 Remove the iPSC from the liquid nitrogen or dry ice and thaw it in water at 37°C. The thawing should be completed quickly;

3.1.2 Disinfect the frozen tube with 75% alcohol and transfer it to bechtop;

3.1.3 Transfer the cell solution to a new 15ml EP tube and flush the primary tube twice with DMEM/F12/DMEM;

3.1.4 Centrifuge the 15ml EP tube at room temperature 300g for 5min(iPSC has good tolerance to 200-300g speed, and 300g is recommended to maximize cell capture, and 200g is recommended in the standard procedure);

3.1.5 Discard the supernatant, gently resuspend the iPSC with 2mL of medium containing ROCK inhibitor, and transfer it to the coated 6-well plate. Shake the plate evenly to distribute the cells (Cell density was adjusted to 1×10^6 cells per well);

3.1.6 Put the 6-well plate back into the 37°C incubator (please do this immediately after the cells are transferred to avoid increased cell center density).

3.1.7 The ROCK inhibitor was removed the next day, and the cells were cultured with the non-inhibitor medium.

Note: The use of antibiotics in cell culture is not recommended as they can interfere with cells and their differentiation potential. The culture environment should be isolated from other cells, and the mycoplasma should be detected after two passages; If the cryopreservation solution contains DMSO, It's toxic to cells at room temperature and the cell thawing procedure should be completed quickly.

3.2. iPSC passaging

3.2.1 Discard the culture supernatant, rinse with 1mL PBS, and add 1mL Acuutase;

3.2.2 Transfer the plate to a 37°C incubator for 3 minutes, or observe under a microscope until most cells fall off (if cells are still attached, place the culture plate in your hand and gently place the other hand on the flap which causes the plate to vibrate slightly to make the cells fall off);

3.2.3 Prepare the coated 6-well plate before passage;

3.2.4 Tilt the culture plate and transfer the Accutase solution twice across the surface of the culture layer to separate the clumps and transfer them to the centrifuge tube;

3.2.5 Rinse the surface of the culture layer with DMEM/F12 and merge with the cell solution in the tube (wash with DMEM/F12 or PBS, at least 5% is recommended Medium for subsequent granulation and attachment);

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

3.2.7 Discard the supernatant and resuspend the cells with a medium containing ROCK inhibitor;

3.2.8 Transfer it to the coated 6-well plate. Shake the plate evenly to distribute the cells;

3.2.9 Put the 6-well plate back into the 37°C incubator for incubation;

Note: iPSCs will rapidly differentiate and die when they grow to a single layer. In order to maintain growth and pluripotency, they should be passed before full growth.

3.3. iPSC cryopreservation

3.3.1 Prepare the cell dissociation solution.

3.3.2 Cell isolation method is similar to the passage, cell counter can be used to cooperate with cell cryopreservation;

3.3.3 Each tube of cells was frozen at the density of 1×10^6, followed by centrifugation, extraction of the medium, and then resuspended in an appropriate volume of frozen solution;

3.3.4 Add 1mL of the resuspended cell cryoprecipitate to the 1.5mL freezing tube, undergo programmed cooling, and store in the liquid nitrogen for a long time.

Disclaimer

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