HC Basement Membrane Matrix, Growth Factor Reduced

(For in vivo experiments, PDX and CDX)

Cat #: A-MGL21

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.

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

Intended Use

This series is divided into standard high concentration and low factor high concentration, their concentration and viscosity are higher than the ordinary concentration of matrix glue, which is more suitable for in vivo experiments (animal models), such as PDX, CDX, in vivo angiogenesis assays.

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.

Example for Procedures, Subcutaneous tumor formation in mice

1. Equipment, reagents and consumables

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

1.2. Reagents: Basement Membrane Matrix (Cat: A-MGL24 / A-MGL26 / A-MGL23 / A-MGL21), basic medium, medium containing 10% fetal bovine serum, 1×PBS, Trypsin Solution

1.3. Consumables: sterile pipette tips; 10cm cell culture dish; Sterile EP tube; Disposable syringes and other consumables can be adjusted according to the experimental design

2. Experimental contents and methods

2.1. Put the Basement Membrane Matrix in the ice box and put it in the refrigerator at 4°C so that the glue can slowly melt overnight;

2.2. Select cells in good condition and growth logarithmically, discard the supernatant, add 2 mL 1×PBS solution to wash gently, discard the liquid;

2.3. Add 1 mL 0.25% trypsin cell digestion solution into the petri dish, let it stand for 10 seconds, discard the trypsin, and continue digestion at room temperature for 1~3 minutes with the residual trypsin.

2.4. When the cells become round (keep in mind that they cannot be digested until the cell edge is clear), add 1 mL medium containing 10% fetal bovine serum to terminate digestion. Carefully blow the cells apart and collect the cell suspension into a 15 mL plastic centrifuge tube.

2.5. Centrifuge at 1000 rpm for 3 minutes and discard the supernatant. After washing the cells with PBS for one time, the cells were re-suspended by adding serum-free basal medium, and 10 μL cell suspension was taken for cell count.

2.6.

HepG2 cells: Each mice should be vaccinated 5 million cells. According to cell density, the required cell suspension was placed in a sterile 1.5 mL plastic centrifuge tube at 1000 rpm for 3 min. The supernatant was discarded and the serum-free medium or PBS was added until the total volume was 300 μL.

HCT - 116 cells: Each mice should be vaccinated 1 million cells. According to cell density, the required cell suspension was placed in a sterile 1.5 mL plastic centrifuge tube at 1000 rpm for 3 min. The supernatant was discarded and the serum-free medium or PBS was added until the total volume was 300 μL.

MIA - PaC- 2 cells: Each mice should be vaccinated 10 million cells. According to cell density, the required cell suspension was placed in a sterile 1.5 mL plastic centrifuge tube at 1000 rpm for 3 min. The supernatant was discarded and the serum-free medium or PBS was added until the total volume was 500 μL.

2.7. Mixing of the Basement Membrane Matrix: Cell suspension and Basement Membrane Matrix are mixed in a 1:1 ratio at 4°C.

2.8. Subcutaneous injection: The nude mice were fixed with the left hand and injected subcutaneously into the right back of the nude mice. During inoculation, the needle was inserted a little deeper into the subcutaneously, about 1 cm deep, to reduce the overflow of cell suspension from the eye after injection, and the inoculation volume was 100 μL.

MIA-PaC-2 cells were injected 200 μL / piece.

2.9. Recording data: The nude mice were put back into the cage for further feeding, the tumor volume was measured regularly according to the experimental requirements, the data was recorded to make a curve, and the nude mice were euthanized before the tumor volume was less than 2000 mm^3, and the tumor was removed and photographed.

Disclaimer

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