Product Name

Name:	mSRCgel™ Extracellular Matrix, High Protein Concentration extracted from EHS mouse sarcoma [-] Phenol Red
Cat. No.:	C2031-0005, C2031-0010
Size:	5 mL, 10 mL

Product Description

The extracellular matrix (ECM) can provide structural support for cells and tissues in a dynamic three-dimensional network of macromolecules. The ECM is a molecular network which holds bioactive molecules and growth factors together. It is of vital importance that it controls the basal behaviors and characteristics of cells such as adhesion, migration, polarity, differentiation, proliferation, and apoptosis.

mSRCgel is a natural basement membrane (BM) extracted from **m**ouse **s**a**rc**oma cells, a type of connective tissue tumor. mSRCgel is high in ECM proteins, including laminin, collagen IV, heparan sulfate proteoglycan (perlecan), entactin, and many essential growth factors.

Application

In vivo angiogenesis studies and 3D tumor models.
Protein concentration: 16 - 26 mg/mL

Storage and Stability

The product should be kept at **-20°C**. Avoid multiple freeze-thaws.
The product is **light-sensitive** and therefore should not be left in the light.
Shelf life: Stable until expiry date on the label

Procedure

During the thawing process, store he mSRCgel Extracellular Matrix at 2 - 8°C overnight. Thawed mSRCgel solidifies quickly above 15°C; when ready to use mSRCgel, keep it on ice to prevent untimely gelling.

Different thicknesses and concentrations are suited to different applications of mSRCgel. A thick gel is needed for applications such as endothelial cell formation of capillary-like structures, epithelial organoid formation, or tumor organoid formation. Some applications require a thin layer coating but not a thick gel, such as propagation of primary cells.

Thick Gel Method:

1. Thaw mSRCgel as described above.
2. Slowly pipet up and down to mix the mSRCgel solution well and do not introduce air bubbles.
3. Pipette 200 - 300 µL/cm^2 mSRCgel solution onto the growth surface.
4. Place the coated plate at 37°C for 30 minutes to solidify.
5. Coated plates are ready for use.

Thin Layer Method (non-gelling):

1. Thaw mSRCgel as described above.

2. Slowly pipet up and down to mix the mSRCgel the solution and do not introduce air bubbles.
3. Dilute mSRCgel to a desired concentration in a cold serum-free medium. A 1: 100 dilution is appropriate for propagating primary cells. An appropriate concentration should be tested out according to the application.
4. Cover the growth surface area with enough solution. Generally, a volume of 300 μL/cm^2 is suitable.
5. Incubate the coated plate at room temperature for one hour.
6. Aspirate the coating solution and plate cells at once. Prevent the coated surface from drying 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, and inverted microscope.

1.2. Reagents: mSRCgel, basal medium, a medium containing 10% fetal bovine serum, 1×PBS, trypsin solution.

1.3. Consumables: sterile pipette tips; 10 cm cell culture dishes; sterile microcentrifuge tubes; the amounts of disposable syringes and other consumables may be adjusted according to the experimental design.

2. Experimental contents and methods

2.1. Put mSRCgel in an ice box and put it (in the refrigerator) at 4°C so that the gel can slowly melt overnight;

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

2.3. Add 1 mL of 0.25% trypsin solution into the petri dish, let it stand for 10 seconds, discard the trypsin, and continue the 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 of the medium containing 10% fetal bovine serum to terminate the digestion. Carefully pipette the cells and collect the cell suspension into a 15 mL plastic centrifuge tube.

2.5. Centrifuge at 1,000 rpm for 3 minutes and discard the supernatant. After washing the cells with PBS once, re-suspend the cells by with serum-free basal medium, and take 10 μL of the cell suspension for cell count.

2.6. HepG2 cells: Each mouse should be inoculated with 5 million cells. The required volume of the cell suspension is centrifuged in a sterile 1.5 mL plastic centrifuge tube at 1,000 rpm for 3 min. The supernatant is discarded and serum-free medium or PBS is added until the total volume is 300 μL.

HCT - 116 cells: Each mouse should be inoculated with 1 million cells. The required volume of the cell suspension is centrifuged in a sterile 1.5 mL plastic centrifuge tube at 1,000 rpm for 3 min. The supernatant is discarded and serum-free medium or PBS is added until the total volume is 300 μL.

MIA - PaC - 2 cells: Each mouse should be inoculated with 10 million cells The required volume of the cell suspension is centrifuged in a sterile 1.5 mL plastic centrifuge tube at 1,000 rpm for 3 min. The supernatant is discarded and serum-free medium or PBS is added until the total volume is 500 μL.

2.7. Mixing with mSRCgel: The cell suspension and mSRCgel are mixed in a 1:1 ratio at 4°C.

2.8. Subcutaneous injection: The left hand of a nude mouse is fixed and the cell suspension is injected subcutaneously into the right back of the nude mice. During the inoculation, the needle is inserted a little deeper, about 1 cm deep, to reduce the overflow of cell suspension from the site of injection, and the inoculation volume is 100μL. For MIA-PaC-2 cells, the volume injected is 200 μL/piece.

2.9. Recording data: The nude mice are put back into the cage for further feeding, and the tumor volume is measured regularly according to the experimental design. The data are recorded to draw a curve and the nude mice are euthanized after the tumor volumes grow to just less than 2,000 mm^3. The tumors is then removed and photographed.

Quality Control

mSRCgel Extracellular Matrix is tested for the presence of bacteria, fungi, and mycoplasma. In addition, osmolality, protein concentration, endotoxin, gel stability, and biological activity are tested.

Precaution and Disclaimer

For research use only, not for clinical diagnosis, and treatment.