Product Name

Name:	mSRCgel Extracellular Matrix extracted from EHS mouse sarcoma [+] Phenol Red
Cat. No.:	C2040-0005, C2040-0010
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

Stem cell cultures, providing the repeatability and consistency required for trophoblastic cultures of human embryonic stem cells and induced pluripotent stem cells.

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

For iPSCs

1. Materials

- Reagents: mSRCgel, ROCK inhibitor, DMEM/F12, mTeSR1/E8 medium, Recombinant Trypsin Solution(C3536), PBS (DPBS).
- Consumables: sterile tips, 6-well plates, sterile microcentrifuge tubes.

2. Preparation

2.1 Materials pre-cooling

- Put mSRCgel in the ice box and put it (in the refrigerator) at 4°C, so mSRCgel 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.
- Supplies or reagents that come into contact with mSRCgel, such as sterile centrifuge tubes, sterile tips, and DMEM/F12, should be pre-cooled at 4°C in advance.

2.2 Dilute mSRCgel at a volume ratio of 1:80 to 1:100

(Note: diluting at 1:80 to 1:100 is all appropriate, equivalent to the mSRCgel concentration at ~0.1 mg/mL. For iPSC culture, coat at a concentration of approximately 0.013 mg/cm², for example, after diluting 12.5 mg/mL of mSRCgel at 1: 100, the coating volume of each 6-well plate is 1 mL)

- Transfer ~ 25 mL of DMEM/F12 at 4°C into the cooled centrifuge tubes;
- Use a pipette with pre-cooled tips to transfer 1 mL of DMEM/F12 to the microcentrifuge tubes with mSRCgel, mix well, and then transfer to the other microcentrifuge tubes (kept on ice).
- The mixture is for subsequent coating.

2.3 Plate coating procedure

- Add 1 mL/well of the mSRCgel mixture into the pre-cooled 6-well plate, and gently shake the plate to ensure that the mixture is evenly spread on the plate;
- Incubate the 6-well plates at 37°C overnight;

  The plates can be used after incubating for 1 - 2 hours, but overnight incubation is better for cell culture. Plates with the coating solution can be stored at 4°C and should be used within one week.
- Remove the coating solution before use.

2.4 Preparation of the ROCK inhibitor working solution: use sterile PBS to dissolve ROCK inhibitor to prepare a stock solution of 10 mM (1000 X) and the working concentration is 10 μM.

2.5 Preparation of medium containing ROCK inhibitor: 10 mM ROCK inhibitor is diluted with mTeSR1/E8 medium to the final concentration of 10 μM.

Note: The mSRCgel at room temperature will gradually polymerization into gel. Please strictly control the operating time to maintain the temperature.

3. Cell culture

3.1 Thawing iPSC

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

  Note: If the cryopreservation solution contains DMSO, it is toxic to the cells at room temperature and the cell thawing procedure should be completed quickly.
- Disinfect the frozen tube with 75% alcohol and transfer it to a biosafety cabinet;
- Transfer the cell solution to a new 15 mL conical tube and wash the primary tube twice with DMEM/F12 (combine all the liquid before the next step);
- Spin the 15 mL centrifuge tube at 300 x *g* for 5 min at room temperature (iPSC has good tolerance to 200 - 300 x *g*, and 300 x *g* is recommended to maximize cell capture, and 200 x *g* is recommended for standard procedure);
- Discard the supernatant, gently resuspend the cells with 2 mL of medium containing ROCK inhibitor, and transfer the cells to the coated 6-well plate. Shake the plate to distribute the cells evenly (The cell density is adjusted to 1×10^6 cells per well);
- Return the 6-well plate back to the 37°C incubator;
- Remove the medium containing ROCK inhibitor the next day, and change to the medium without ROCK inhibitor .

Note: The use of antibiotics in cell culture is not recommended as they can interfere with the cells and their differentiation potentials.

3.2 Passaging iPSC

- Discard the supernatant, rinse with 1 mL of PBS, and add 1 mL of 0.05% EDTA solution (C3550-0100);
- Transfer the plate to a 37°C incubator for 3 - 6 minutes, or observe the plate under a microscope until most of the cells fall off (Tapping helps to dissociate the cells to fall off from the plate);
- Prepare the coated 6-well plate before passage;
- Tilt the culture plate and pipette the solution to rinse over the whole surface of the cell layer twice to dissociate the cells and transfer them to a centrifuge tube;
- Rinse the surface of the plate with DMEM/F12 and combine the cell solution in the tube (wash with DMEM/F12 or PBS);
- Centrifuge the tube at 300 x *g* at room temperature for 5 min;
- Discard the supernatant and resuspend the cells with the medium containing ROCK inhibitor;
- Transfer it to the coated 6-well plate. Shake the plate to distribute the cells evenly;
- Put the 6-well plate back into the 37°C incubator.

Note: iPSCs will rapidly differentiate and die when they grow to confluence. To maintain growth and pluripotency, they should be passaged before reaching confluence.

3.3 iPSC cryopreservation

- Dissociate the cells from the plate and count the cell number;
- Pipette 1 x 10^6 cells to each cryogenic tube, spin down the cells at 300 x *g*, remove the medium, and then resuspend the cells in an appropriate volume of freezing medium;
- Add 1 mL of the cells in freezing medium to the 1.5 mL freezing tube, store the tubes at -80°C, and transfer to liquid nitrogen tank for long-term storage.

Precaution and Disclaimer

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