StemFit® For MSC with Synthemax/Vitronectin Protocol

Materials Required

- Cryopreserved Mesenchymal stem cells (MSCs)
- StemFit® For MSC (AMSBIO #SFMSC-A3)
- Synthemax II (Corning #3535) or Vitronectin-C (Vitronectin 20-398 aa)
- Cell detachment solution (e.g. Detachin (AMSBIO #AMS.T100100), TrypLE™ Select (Thermo Fisher) or Accumax (MERCK Millipore))
- PBS (AMSBIO #2113-500)

Using ECM-Coated Culture Vessels

1. Coat the plate/dish with Synthemax II (5 µg/cm^2) or vitronectin (0.5 µg/cm^2).
2. Add 9 mL of StemFit® For MSC into a polypropylene (PP) conical tube.
3. Quickly thaw the cryovial in a 37°C water bath within 2 min. Stop warming when the last piece of ice remains.
4. Transfer cell suspension from cryovial into the conical tube prepared in step 2.
5. Centrifuge at 500 x g for 5 min at room temperature. Decelerate without the use of an applied brake.
6. Aspirate the supernatant. Tap the tube to loosen the pellet and resuspend the cells with StemFit® For MSC medium.
7. Determine the cell concentration.
8. Seed cells at 5,000-10,000 cells/cm^2 in StemFit® For MSC medium. E.g:
    - 6 well plate: 5.0 x 10^4 cells / 2 mL / well
    - T75 flask: 3.8 x 10^5 cells / 10-15 mL
9. Culture cells at 37C, 5% CO_2. Change the medium once in 2-3 days.
    - **Note: Synthemax II/Vitronectin-C is not required except for replating the cells after passage.**
10. Subculture when cells are approximately 70-90% confluent.

Using Non-Coated Vessels

1. Prepare StemFit® For MSC + Synthemax medium by adding Synthemax II (1 mg/mL) to StemFit® For MSC medium to a final concentration of 1 µg/mL.
    - E.g. add 10 µL of 1 mg/mL Synthemax II into 10 mL StemFit® For MSC.
2. Add 9 mL of "StemFit® For MSC + Synthemax medium" prepared in step 1 into a conical tube.
3. Quickly thaw the cryopreserved MSC cryovial into the conical tube prepared in step 2.
4. Centrifuge at 500 x g for 5 min at room temperature. Decelerate without the use of an applied brake.
5. Aspirate the supernatant. Tap the tube to loosen the pellet and resuspend the cells with StemFit® For MSC medium.
6. Determine the cell concentration.

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, U.K.
T: +44 (0) 1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141.
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Via Lisano 3
(CP.683)
6900 Massagno
T: +41 (0) 91 604 55 22
F: +41 (0) 91 605 17 85

7. Seed cells at 5,000-10,000 cells/cm^2 in StemFit® For MSC medium. E.g.:
    - 6 well plate: 5.0 x 10^4 cells / 2 mL / well
    - T75 flask: 3.8 x 10^5 cells / 10-15 mL
8. Culture cells at 37C, 5% CO_2. Change the medium once in 2-3 days.
    - **Note: Synthemax II/Vitronectin-C is not required except for replating the cells after passage.**
9. Subculture when cells are approximately 70-90% confluent.

Cell Expansion

1. Prepare "StemFit® For MSC + Synthemax medium" by adding Synthemax II (1 mg/mL) to StemFit® For MSC medium to a final concentration of 1 µg/mL.
    - E.g. add 10 µL of 1 mg/mL Synthemax II into 10 mL StemFit® For MSC.
2. Aspirate the medium and wash with PBS.
3. Add cell detachment solution (e.g. Detachin (AMSBIO #AMS.T100100), TrypLE™ Select (Thermo Fisher) or Accumax (MERCK Millipore)) E.g.:
    - 6 well plate: 500 µL / well
    - T75 flask: 4 mL/ flask
4. Incubate at 37°C for 10 min until all cells are rounded and dissociation of cells is apparent.
5. Pipette the cells in the Cell Detachment Solution to fully dissociate cells and transfer to a PP conical tube.
6. To collect cells remaining in the vessel, add "StemFit® For MSC + Synthemax medium" to the well/flask and then transfer to the conical tube.
    - 6 well plate: 1 mL / well
    - T75 flask: 8 mL / flask
7. Centrifuge at 200 x g for 5 min at room temperature.
8. Aspirate the supernatant completely.
    - **Note: Eliminate dissociation reagent completely. Remaining dissociation reagent may inhibit cell attachment to culture vessel.**
9. Tap the tube to loosen the pellet and resuspend the cells with 0.5-1 mL "StemFit® For MSC + Synthemax medium".
    - **Note: Please adjust the volume of medium according to culture scale.**
10. Determine the cell concentration.
11. Seed cells at 5 x 10^3 cells/cm^2 in StemFit® For MSC medium. E.g:
    - 6 well plate: 5.0 x 10^4 cells / 2 mL / well
    - T75 flask: 3.8 x 10^5 cells / 10-15 mL
12. Culture cells at 37°C, 5% CO_2. Change the medium once in 2-3 days.
    - **Note: Synthemax II/Vitronectin-C is not required except for replating the cells after passage.**
13. Subculture when cells are approximately 70-90% confluent.
    - **Note: Do not allow cells to become over confluent since it will be difficult to detach and collect cells.**