

## Isolation of mesenchymal stem cells from bone marrow mononuclear cells (BMMNCs) using StemFit® For MSC

### Materials Required

- Bone marrow mononuclear cells
- StemFit® For MSC (AMSBIO #SFMSC-A3)
- Synthemax II (Corning #3535) or Vitronectin-C (Vitronectin 20-398 aa)
- iMatrix-511 (AMSBIO #AMS.892 012, #AMS.892 011)
- PBS (AMSBIO # 2113-500)

### Method

1. Coat the plate/dish with Synthemax II (5 µg/cm<sup>2</sup>) or Vitronectin-C (1.5 µg/cm<sup>2</sup>)
2. Obtain fresh mononuclear cells (MNC) from human bone marrow (BM) by density centrifugation (Ficoll-Paque) or thaw the frozen BMMNC. Determine the cell concentration.
3. Seed BMMNC at 1.3 x 10<sup>6</sup> cells/cm<sup>2</sup> in StemFit® For MSC medium. E.g.:
  - 24-well plate: 6 x 10<sup>6</sup> cells / 0.5 mL / well
  - 6-well plate: 1.3 x 10<sup>7</sup> cells / 2 mL / well
  - 60 mm dish: 2.7 x 10<sup>7</sup> cells / 5 mL / dish
4. Culture the cells at 37°C, 5% CO<sub>2</sub>.
5. Change the medium the next day.
6. Add 20% medium on day 3 and 5 after seeding.
7. 7 days after seeding, replate all the cells in the vessel according to the procedure below.
8. Prepare “StemFit® For MSC + iMx” medium by adding iMatrix-511 (0.5 mg/mL) to StemFit® For MSC medium to a final concentration of 0.2 µg/mL.
  - E.g add 4 µL of 0.5 mg/mL iMatrix-511 into 10 mL StemFit® For MSC.
  - Note: Users can use appropriate ECM-coated plate instead of adding iMatrix-511 into the medium.
9. Collect the culture supernatant in polypropylene (PP) conical tube.
10. Add PBS, pipette the cells 10 times to fully dissociate cells and transfer them to the conical tube.
11. Centrifuge at 200 x g for 5 min at room temperature. Decelerate without the use of an applied brake.

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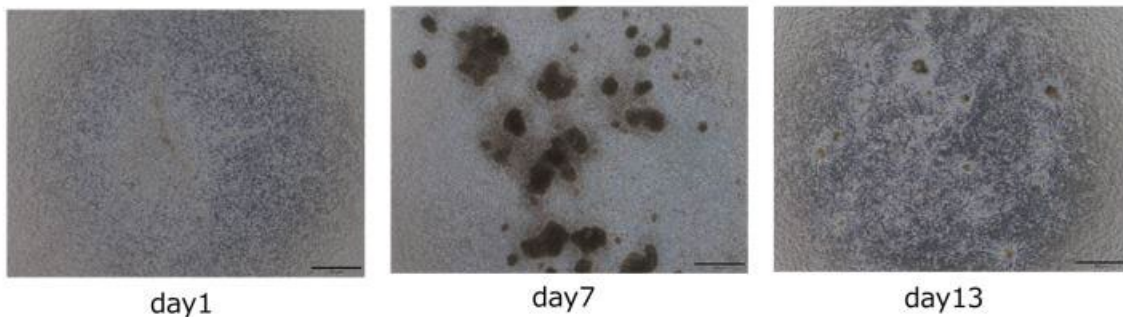
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12. Aspirate the supernatant completely
13. Tap the tube to loosen the pellet and resuspend the cells in the StemFit® For MSC + iMx medium.
14. Seed all the cells in “StemFit® For MSC + iMx” medium.
15. Change the medium on day 3 after replating. Following this, change the medium once in 2-3 days.
16. Subculture when cells are approximately 70-90% confluent.



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