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AJINOMOTO

StemFit

Product Information: StemFit For Mesenchymal Stem Cell

1. Introduction

StemFit For Mesenchymal Stem Cell (StemFit For MSC) is a chemically defined medium for mesenchymal stem cell (MSC) culture. This medium does not contain any undefined components such as serum or human platelet lysate, and it enables the maintenance of human bone marrow-derived MSC (BM-MSC), umbilical cord-derived MSC (UC-MSC) and adipose-derived stem cells (ADSC), under chemically defined conditions.

2. Materials Provided

Volume	Storage
500 ml	Store at -20 °C

Note: Add scaffold protein (e.g. iMatrix-511, Vitronectin)

3. Media Preparation

Use sterile techniques to prepare StemFit For MSC medium.

1) Thaw StemFit medium at room temperature (15-25 °C) or at 2-8°C overnight. Mix thoroughly.
   CAUTION: Do not thaw StemFit For MSC at 37 °C, as it accelerates medium degradation
   > Thawed StemFit For MSC medium may be stored at 2-8 °C for up to a month. ***Protect from light.***
   > Optionally, the medium can be stored as aliquots at -20°C until the expiration date. Do not re-freeze thawed aliquots.
2) Warm medium to room temperature and use immediately.

Key points for successful culture

Do not thaw the media at 37°C because it accelerates degradation of media.

Add iMatrix-511 or use matrix coated plate.

Remove dissociation reagent completely after centrifugation.

Avoid over confluent culture because that makes it difficult to detach and collect cells.

4. Thawing Protocol of Cryopreserved MSC

A) Use of Non-Coated Vessels

1. 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.
   > **Example**: Add 4 µL of 0.5 mg/mL iMatrix-511 into 10 mL StemFit For MSC.
2. Add 9 mL of "StemFit For MSC + iMx medium" prepared in step 1 into a conical tube.
3. Quickly thaw the cryopreserved MSC cryovial in a 37 °C water bath within 2 min. Stop warming when the last piece of ice remains.
4. Transfer the cell suspension from the 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 + iMx medium".
7. Determine the cell concentration.
8. Seed cells at 5,000-10,000 cells / cm^2 in "StemFit For MSC + iMx medium".
   > **Example:** 5.0 x 10^4 cells / 2 mL / well in 6 well plate.
   > **Example:** 3.8 x 10^5 cells / 10-15 mL in T75 flask.
9. Continue to **C)**

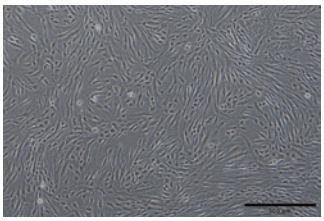
B) When Using ECM-Coated Culture Vessels

iMatrix-511, Fibronectin, or Vitronectin-coated plates are also compatible for use with StemFit For MSC.

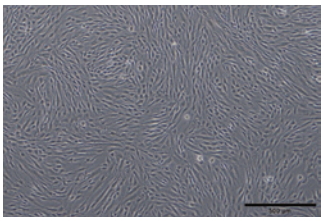
1. Add 9 mL of StemFit For MSC into a polypropylene (PP) conical tube.
2. Quickly thaw the cryovial in a 37 °C water bath within 2 min. Stop warming when the last piece of ice remains.
3. Transfer cell suspension from cryovial into the conical tube prepared in step 1.
4. Centrifuge at 500 x g for 5 min 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.
7. Seed cells at 5,000-10,000 cells / cm^2 in StemFit For MSC medium.
   > **Example**: 5.0 x 10^4 cells / 2 mL / well in 6 well plate. > **Example**: 3.8 x 10^5 cells / 10-15 mL in T75 flask.
8. Continue to **C)**

C) Culture for both A) and B) (Culture for Both A and B)

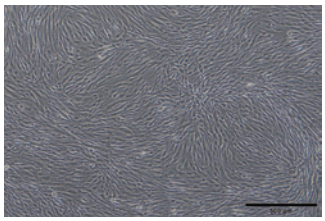
1. Culture cells at 37°C, 5% CO_2
2. Change the medium once in 2-3 days.
   Note: iMatrix-511 is not required except for re-plating cells after passage.
3. Subculture when cells are approximately 70-90% confluent.
   Caution: Do not allow cells to become over confluent as shown in (c), since it will be difficult to detach and collect cells. Passage should be done while cells are between conditions shown in (a) and (b).



(a) 70% confluent



(b) 90% confluent



(c) over confluent

5. Cell Expansion

1. 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.
   > **Example**: Add 4 µL of 0.5mg/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. iMatrix-511, Fibronectin, or Vitronectin-coated plates are compatible for use with StemFit For MSC.
2. Aspirate the medium and wash once with PBS.
3. Add Cell Detachment Solution [e.g. TrypLE™ Select (Thermo Fisher) or Accumax (MERCK Millipore)]
   > **Example**: 500 µL / well for 6 well plate. > **Example**: 4 mL / flask for T75 flask.
4. Incubate at 37°C for 10 mins 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 + iMx medium" to the well / flask and then transfer to the conical tube.
   > **Example**: 1 mL / well for 6 well plate. > **Example**: 8 mL / flask for T75 flask.
7. Centrifuge at 200 x g for 5 min at room temperature.
8. Aspirate the supernatant completely.
   Caution: 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 + iMx medium".
   Note: Please adjust the volume of medium according to the culture scale.
10. Determine the cell concentration.
11. Seed the cells at 5.0 x 10^3 cells / cm^2 in "StemFit For MSC + iMx medium".
    > **Example**: 5.0 x 10^4 cells / 2 mL / well in 6 well plate. > **Example**: 3.8 x 10^5 cells / 10-15 mL in T75 flask.
    Note: Users can use matrix-coated plate instead of adding iMatrix-511 into the media.
12. Culture the cells at 37°C, 5% CO_2.
13. Change the medium once in 2-3 days.
    Note: iMatrix-511 is not required except for re-plating cells after passage.
14. Subculture when cells are approximately 70-90% confluent.
    Caution: Do not allow cells to become over confluent since it will be difficult to detach and collect cells. Passage should be done while cells are between conditions shown in (a) and (b) of step 3 in chapter 4 part C (Thawing protocol of cryopreserved MSC).

6. Precaution and Disclaimer

StemFit For MSC is for research use only and is not intended for human or animal diagnostic or therapeutic uses.

7. Contact the following department for product information:

Amino Acid Dept., AminoScience Division, AJINOMOTO CO., INC.
1-15-1 Kyobashi, Chuo-ku, Tokyo 104-8315, Japan E-mail: **stemfit@ajinomoto.com**

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