1. Introduction

StemFit For Mesenchymal Stem Cell (StemFit For MSC) is an animal origin-free, chemically defined medium for mesenchymal stem cell (MSC) culture. This medium does not contain any human and animal-derived components 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 serum-free, human platelet lysate-free conditions. This medium cannot be used for isolation of MSC.

2. Materials Provided

<table>
<thead>
<tr>
<th>Volume</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 ml</td>
<td>Store at -20 °C</td>
</tr>
</tbody>
</table>

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.

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. 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 / cm² in “StemFit For MSC + iMx medium”.
   Example: 5.0 x 10⁴ cells / 2 mL / well in 6 well plate.
   Example: 3.8 x 10⁵ cells / 10-15 mL in T75 flask.

9. Continue to C)

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. 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 / cm² in “StemFit For MSC + iMx medium”.
   Example: 5.0 x 10⁴ cells / 2 mL / well in 6 well plate.
   Example: 3.8 x 10⁵ cells / 10-15 mL in T75 flask.

9. Continue to C)
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.
   \[ \text{Example: Add 4 µL of 0.5mg/mL iMatrix-511 into 10 mL StemFit For MSC.} \]

2. Aspirate the medium and wash once with PBS.

3. Add Cell Detachment Solution [e.g. TrypLE™ Select (Thermo Fisher) or Accumax (MERCK Millipore)]
   \[ \text{Example: 500 µL / well for 6 well plate.} \]
   \[ \text{Example: 4 mL / flask for T75 flask.} \]

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.
   \[ \text{Example: 1 mL / well for 6 well plate.} \]
   \[ \text{Example: 8 mL / flask for T75 flask.} \]

7. Centrifuge at 200 x g for 5 min at room temperature.

Caution: Eliminate dissociation reagent completely. Remaining dissociation reagent may inhibit cell attachment to culture vessel.

8. Aspirate the supernatant completely.

9. Tap the tube to loosen the pellet and resuspend the cells with 0.5-1 mL “StemFit For MSC + iMx medium”.

10. Determine the cell concentration.

11. Seed the cells at 5.0 x 10^3 cells / cm² in “StemFit For MSC + iMx medium”.
    \[ \text{Example: 5.0 x 10^4 cells / 2 mL / well in 6 well plate.} \]
    \[ \text{Example: 3.8 x 10^5 cells / 10-15 mL in T75 flask.} \]

12. Culture the cells at 37°C, 5% CO₂.

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).

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. 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 / cm² in StemFit For MSC medium.
   \[ \text{Example: 5.0 x 10^4 cells / 2 mL / well in 6 well plate.} \]
   \[ \text{Example: 3.8 x 10^5 cells / 10-15 mL in T75 flask.} \]

8. Continue to C)

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

1. Culture cells at 37°C, 5% CO₂.

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).

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:

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