工業技術研究院
Industrial Technology
Research Institute

SF1 hMSC Phenol Red Free Medium

Product Information Sheet

Introduction

SF1 hMSC Phenol Red Free Medium is a phenol red free, serum free and animal-component free cell culture medium. This medium is designed for the growth and expansion of human mesenchymal stem cells (MSCs) derived from bone marrow, adipose or Wharton's Jelly. Using SF1 hMSC phenol red free Medium, human MSCs can be expanded quickly for multiple passages while maintaining their ability to differentiate into osteogenic, chondrogenic and adipogenic lineages.

Plate-coating is not needed for the SF1 hMSC phenol red free Medium, which means that the user can use tissue culture vessels directly and save labor and cost related to coating plate. Information of recommended tissue culture vessels is provided in this sheet.

Cat. No.：MSC-SF-006 includes:

Item	Product	Cat. No	Amount	Storage
1	hMSC SF1 Basal Medium Phenol Red Free	MSC-SF-007	450 mL	2 to 8℃, **must in the dark**
2	hMSC SF1 Supplement Phenol Red Free	MSC-SF-008	50 mL	-20℃, **must in the dark**

Note:

1. SF1 hMSC Medium is sold as a complete kit; its components are not available separately.
2. SF1 hMSC Phenol Red Free Medium is very sensitive with light. Store in the dark is very important.

Intended Use

SF1 hMSC Phenol Red Free Medium is intended for human *ex vivo* tissue and cell culture processing applications.

CAUTION: Not intended for direct administration to humans or animals.

Precaution:

Please read the prepared complete medium procedure before use.

1. Thaw the frozen hMSC SF1 Supplement Phenol Red Free at 4℃ refrigerator about 12-20 hrs prior use, do not over 24 hrs.
2. Swirl the bottle on the desk gently to mix the hMSC SF1 Supplement Phenol Red Free solution.
3. Warm the hMSC SF1 Basal Medium Phenol Red Free (Cat. No MSC-SF-007) in a 37℃ water bath for 25-30 min.

4. Warm the hMSC SF1 Supplement (Cat. No MSC-SF-008) in a 37℃ water bath for 10-15 min. (The warming times please do not longer than 20 min.)
5. Swirl warmed hMSC SF1 Supplement Phenol Red Free and hMSC SF1 Basal Medium Phenol Red Free on the desk gently.
6. Aseptically transferred the entire contains of hMSC SF1 Supplement Phenol Red Free bottle to hMSC SF1 Basal Medium Phenol Red Free bottle.
7. Swirled the hMSC SF1 Basal Medium Phenol Red Free bottle gently to ensure complete mixing. **This is the complete medium**.
8. Rinse the hMSC SF1 Supplement Phenol Red Free bottle with complete medium for 3 times, about 30 to 35 mL per time.
9. Swirl the SF1 hMSC Phenol Red Free Medium bottle gently to ensure complete mixing.
10. Please aliquot the complete medium if you do not once use the medium.
11. Please use the aliquot medium within two months.

Cell Culture Notes and Procedure

- **Tissue culture vessels**

1. We recommend use Corning CellBind® product line of culture vessels. Corning/COSTAR general type tissue culture product, BD Primaria® product line and Falcon plates (6, 12 well-plate etc.) for MSCs cultured in SF1 hMSC Medium.
2. We highly recommend use Corning CellBind® products for initial plating of primary isolated Mesenchymal Stem Cells from bone marrow, adipose or Wharton's Jelly (P0). Corning CellBind® products also are the best choice for the low density seeding related experiment (for example, Colony Forming Unit Assay, seeding density below $5x10^2/cm^2$).

- **Culture procedure**

The following protocols have been used successfully in our laboratories.

1. It's highly recommend to **warm the SF1 hMSC Phenol Red Free Medium in 37℃ water bath for about 15 minutes and gently mix it** before the experiment.
2. Remove media from T-25cm^2 flask. Gently wash flask with 5 mL Dulbecco's PBS (without Ca^{2+} or Mg^{2+}) for about 1 minute and removes the solution.
3. Add 1 mL pre-warmed Trypsin reagent to flask and dispense evenly over the entire culture surface. **We recommend products from Life technologies, TrypLE Select cat. no. 12563** or 0.05% Trypsin-EDTA (0.53mM). But 0.25% Trypsin-EDTA (1mM) is not recommend for the MSCs subculture.
4. Incubate the flask at 37℃ until cells become rounded (about 2-5 minutes) and are easily dislodged from the surface. Firmly tap the flask as necessary to facilitate cell detachment.
5. Once detached, add 6 mL SF1 hMSC Phenol Red Free medium to flask, mix gently and transfer cell suspension to a 15 mL centrifuge tube. **(Note: Don't use Dulbecco's PBS as suspension medium)**
6. Centrifuge for 5 minutes at 290 x g (~1200 rpm for most table-top centrifuges).

7. Remove supernatant and re-suspend cell pellet in 1 mL SF1 hMSC Phenol Red Free Medium.
8. Determine viable and total cell number. Determine cell viability by trypan blue-dye exclusion method, and use a hemocytometer or other counting system (for example, ADAM system or Vi-CELL system) to determine cell number.
9. Seed cell to flasks at 0.5-1x10^3 viable cells/cm^2. The seeding density recommended for MSCs derived from adipose is 0.5x10^3, for MSCs derived from bone marrow or Wharton's Jelly is 1x10^3/cm^2.
10. Incubate cells at 37℃ in a humidified chamber containing 5% CO_2.
11. Re-fresh medium with pre-warmed SF1 hMSC Phenol Red Free Medium after 3 days of culture.
12. Subculture until cells are 80-90% confluent, which takes about 4-6 days after seeding.

- **Cryopreservation of cells with SF1 hMSC Phenol Red Free Medium**
  1. Prepare cryopreservation solution by supplementing SF1 hMSC Phenol Red Free Medium with 10% Dimethyl Sulfoxide (DMSO).
  2. Pellet detached cells by centrifugation (290xg, 5minutes), gently resuspend cells in cryopreservation solution to 0.3-1x10^6 cells/mL, and transfer to cryovials.
  3. Place cryovials in a freezing container (e.g. Nalgene 5100-0001) and place in a -70 to -80°C freezer overnight.
  4. Transfer cryovials to liquid nitrogen for long-term storage.

- **Recovery of Cryopreserved Human MSCs**
  1. Rapidly thaw frozen vial of cells in a 37°C water bath.
  2. Add 8-9 mL pre-warmed (37°C) SF1 hMSC Phenol Red Free Medium in one 15 mL conical tube.
  3. Pipet the entire contents of the cryovial into the conical tube. Tighten the cap and inverse the tube gently to mix the cells and medium.
  4. Follow the "**Culture procedure**" (upper section) step 6 to 12 to seed the cells.

Quality Control Testing of the Lot

The performance of SF1 hMSC Phenol Red Free Medium was verified by cell expansion assay using MSCs derived from adipose. Sterility test and LAL test compliant with USP were performed to verify the absence of endotoxin, bacterial and fungal contamination.

Manufacture

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