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# MesenPlify™ sXF

*Xeno-Free, Serun-Free MSC Expansion Media*

Catalogue Number C0001

**Product Sheet**

**MesenPlify™ sXF** is a **Xeno-free, Coating-free, Serum-free** complete media formulation designed to support the *in-vitro* growth and proliferation of human mesenchymal stem cells (hMSCs). The use of MesenPlify™ sXF demonstrates exceptional capabilities in facilitating the long-term maintenance and proliferation of hMSCs, while preserving their multi-lineage differentiation potential (Osteogenic, Adipogenic, Chondrogenic) and essential cell surface expression markers (CD73, CD90, CD105) as defined by the ISCT minimal criteria.

**MesenPlify™ sXF** has been tested to be highly suitable for culturing a wide range of hMSCs, including Bone Marrow derived (BM-MSC), Adipose derived (AD-MSC) and Umbilical Cord derived (UC-MSCs).

## A. Properties

**MesenPlify™ sXF** is supplemented with human platelet lysate (hPL) from human source, xeno-free alternative to Fetal Bovine Serum (FBS), which is frequently used for growth and expansion of cells. hPL has been shown to have better lot to lot consistency compared to FBS.

## B. Components

**MesenPlify™ sXF** Complete Media (#C0001) consists of 2 components:

| Product | Catalogue Number | Volume | Storage Condition | Shelf Life |
|---|---|---|---|---|
| **MesenPlify™ sXF Basal Media** | S0001 | 470mL | 2 to 8°C | 1 Year from date of manufacture. |
| **MesenPlify™ sXF Supplement** | SU0001 | 30mL | -15°C to -20°C | 1 Year from date of manufacture. |

*NOTE1: None of the above component contain antibiotics.*
*NOTE 2: For complete media, please use it within 2 weeks if stored at 2℃–8℃.*

## C. Materials Required but Not Supplied

- 0.25% Trypsin-EDTA
- D-PBS (without Ca++, without Mg++)
- Culture dish and flasks
- Polypropylene conical tubes

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## D. Safety

Treat all human derived materials as potentially infectious.

Read the product SDS, adhere to BSL-2 practises, wear appropriate personal protective equipment, handle the materials in a certified Biological Safety Cabinet.

## E. Preparation of complete media

Perform following procedures using aseptic techniques to prepare complete media in a biological safety cabinet. The following instruction is for preparing 500mL of complete media.

1. Thaw **MesenPlify™ sXF supplement (Catalogue No. SU0001)** at room temperature or 2-8°C.
   *NOTE: Use immediately after thaw, or aliquot in polypropylene tubes and freeze at -20°C. Do not use beyond the shelf life of individual components. Avoid multiple freeze-thaw cycles of supplement.*
2. Add 30ml of **MesenPlify™ sXF supplement** to 470mL **MesenPlify™ sXF Basal Media (Catalogue No. S0001)** to 500mL complete media. Mix thoroughly.
3. Store the **MesenPlify™ sXF complete media at 2-8°C**, away from light, for up to 2 weeks.
   *NOTE: If desired, an additional filtration step using 0.2um filter units can be performed. If desired, antibiotics can be added at 1% of final complete media volume.*

## F. Guideline for use

### i. Recovery of Cryopreserved hMSCs

1. Pre-warm complete **MesenPlify™ sXF complete media** to 37°C.
2. Rapidly thaw a frozen vial of hMSC in a 37°C water bath until a small amount of ice remains.
3. Transfer the contents of the vial into a 15ml conical tube. Slowly pipet **10ml of MesenPlify™ sXF complete media** dropwise into the conical tube.
4. Centrifuge at **300 x g** for **5 minutes** at room temperature.
5. Discard supernatant to remove DMSO cryoprotectant.
6. Resuspend the cell pellet in **1mL** of **MesenPlify™ sXF** complete media.
7. Perform cell count.

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8. Top up cell suspension with **MesenPlify™ sXF** complete media to an appropriate volume.
   *RECOMMENDATION: Use 30mL of MesenPlify™ sXF media in 150mm dish and 15mL for T75 flask. See below table 1 Recommended Usage of Media and Digestive Enzyme for hMSC culture.*
9. Seed cells in tissue culture treated flasks at **3,000-5,000 cells per cm²**.
   *NOTE: A higher seeding density is recommended when cells are first recovered from cryopreservation.*
10. Culture cells in a humidified $CO_2$ incubator at 37°C, 5% $CO_2$.
11. Perform complete media change **every 3-4 days** with **MesenPlify™ sXF** complete media pre-warmed to 37°C. Passaging is required when confluency reached **70-80%** *(see below section for sub-culture of hMSCs).*

## ii. Sub-culture of hMSCs

1. Check that hMSCs are 70 – 80% confluent. Do not allow hMSCs to reach 100% confluency, which cells would exhibit signs of differentiation.
2. Pre-warm **MesenPlify™ sXF complete media** to 37°C
3. Gently wash cells once with DPBS (without Ca++, without Mg++, not provided).
4. Add sufficient 0.25% Trypsin-EDTA (not provided in the kit) to cover entire surface of tissue culture flask.
   *RECOMMENDATION: Use 3ml of 0.25% Trypsin-EDTA per T75 flask and 5ml of trypsin per 150mm dish.*
5. Swirl flask to spread 0.25% Trypsin-EDTA across surface.
6. Incubate at 37°C for 2-5 mins.
   *NOTE: Exact digestion timing is subject to optimization. Observe detachment under microscope to avoid over-digestion.*
7. Observe cell detachment under microscope. Gently tap the sides of the flask to aid in detachment if necessary.
8. Once cells have detached, add minimum **2x volume of MesenPlify™ sXF complete media** to quench the digestion.
   *RECOMMENDATION: Use 6ml of* complete MesenPlify™ sXF media *per T75 flask and 10ml of* complete MesenPlify™ sXF media *per 150mm dish.*

9. Collect cell suspension in a 15 or 50ml conical tube.
10. Centrifuge at **300 x g** for **5 minutes** at room temperature.
11. Discard supernatant to remove trypsin.
12. Resuspend the cell pellet in **1mL** of **MesenPlify™ sXF** complete media.
13. Perform cell count.
    *NOTE: Sample may need to be further diluted in media if cell density exceeds recommended range of cell counter.*
14. Calculate volume of cell suspension needed to seed at **3,000 - 5,000 cells per cm$^2$.**
15. Top up cell suspension with **MesenPlify™ sXF** complete media to an appropriate volume.
    *RECOMMENDATION: Use 30mL of MesenPlify™ sXF media in 150mm dish and 15mL for T75 flask.*
16. Culture cells in a humidified $CO_2$ incubator at 37°C, 5% $CO_2$.
17. Perform complete media change **every 3-4 days** with **MesenPlify™ sXF** complete media pre-warmed to 37°C. Passaging is required when confluency reached **70-80%.**

### iii. Cryopreservation of hMSCs

1. Prepare 2X cryopreservation media by supplementing 20% of Dimethyl Sulfoxide (DMSO, not provided).
2. Resuspend cell pellet to twice of the desired cell concentration (e.g. 2 x $10^6$ /mL) in pre-warmed complete MesenPlify™ sXF media.
3. Slowly add the 2X cryopreservation media (*prepared in step 1*) to the cell suspension, and gently mix by pipetting.
4. Transfer the cell suspension to pre-chilled (2°C - 8°C) cryovials.
5. Place the cryovials at -80°C in a cryogenic freezing container overnight.
6. Transfer the frozen cells to liquid nitrogen (vapor phase) for long-term storage.

## G. Characterization of hMSC

hMSCs cultured can be characterized with the following assays,

- Flow Cytometry for cell surface marker (refer to guideline from ISCT for MSC surface marker)
- Colony Forming Unit assay for cell proliferation
- Multi-lineage Differentiation assay for differentiation potential.

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## H. Adaptation of hMSC to MesenPlify™ sXF media

In case of using other brands for hMSCs culture, hMSCs can be directly adapted to complete MesenPlify™ sXF media without prior conditioning.

### Table 1. Recommended Usage of Media and Digestive Enzyme for hMSC culture

| Culture ware | Area | MesenPlify sXF complete media | 0.25% Trypsin -EDTA |
|---|---|---|---|
| **6 well plate** | 9.6cm$^2$/well | 1.5mL/well | 0.5mL/well |
| **T75 Flask** | 75cm$^2$ | 15mL | 3mL |
| **150mm Dish** | 145cm$^2$ | 30mL | 5mL |

## Warranties

For Research Use Only (RUO), or for further manufacturing.

Not for Human or Animal Diagnostic or Therapeutic use.

The product is manufactured in accordance with the quality management system related to ISO 9001.