

# MesenPlify™ sXF (500mL)

## Product Sheet

Catalogue Number C0001

### Product Description

**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).

### 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.

### Components

The MesenPlify™ sXF cell culture kit consists of 2 components:

Product	Catalogue Number	Volume	Storage Condition	Shelf Life
<b>MesenPlify™ sXF</b> Xeno Free, Serum-Free Human MSC Expansion <b>Basal Media</b>	S0001	470mL	2 - 8°C	1 Year from date of manufacture
<b>MesenPlify™ sXF</b> Xeno Free, Serum-Free Human MSC Expansion <b>Supplement</b>	SU0001	30mL	-15 - -25°C	1 Year from date of manufacture

None of the above component contain antibiotics.

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### Materials Required but Not Supplied

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

### Safety

Treat all human derived materials (e.g. hMSCs, hPL) 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.

### Preparation of complete media

*(Note: Perform all procedures using aseptic techniques.)*

1. Thaw the 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. Do not re-freeze aliquots.)***

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 complete MesenPlify™ sXF 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.)***

### Guideline for use

#### A. 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.

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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.
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.)**
9. Seed cells in tissue culture treated flasks at **3,000-5,000 cells per cm<sup>2</sup>**.  
**(Note: A higher seeding density is recommended when cells are first recovered from cryopreservation)**
10. Culture cells in a humidified CO<sub>2</sub> incubator at 37°C, 5% CO<sub>2</sub>.
11. Perform complete media change **every 3-4 days** with complete MesenPlify™ sXF media pre-warmed to 37°C. Passaging is required when confluency reached **70-80%** (see below section for sub-culture of hMSCs).

### **B. 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<sup>++</sup>, without Mg<sup>++</sup>, not provided in the kit).
4. Add sufficient **trypsin** (not provided in the kit) to cover entire surface of tissue culture flask.  
**(Recommendation: Use 3ml of trypsin per T75 flask and 5ml of trypsin per 150mm dish).**
5. Swirl flask to spread trypsin 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. Using a phase-contrast microscope, check for cell detachment. Gently tap the sides of the flask to aid in detachment.

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8. Once cells have detached and are free-floating, add **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<sup>2</sup>**.
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<sub>2</sub> incubator at 37°C, 5% CO<sub>2</sub>.
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%**.

### C. Cryopreservation of hMSCs

1. Prepare 2X cryopreservation media by supplementing 20% of Dimethyl Sulfoxide (DMSO, not provided in the kit).
2. Resuspend cell pellet to twice of the desired cell concentration (e.g.  $2 \times 10^6$  /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.

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### Characterization of hMSC

hMSCs cultured can be characterized with flow cytometry, colony forming unit assay, and multi-lineage differentiation assay.

### 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	Trypsin Required
6 well plate	9.6cm <sup>2</sup> /well	1mL/well	0.5mL/well
T75 Flask	75cm <sup>2</sup>	15mL	3mL
150mm Dish	145cm <sup>2</sup>	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.

Disclaimer: While InnoCellular Tech has taken all reasonable measure to ensure the accuracy and correctness of the information provided, it does not provide any warranties concerning the accuracy of such information. InnoCellular warrants its products meet the applicable standards as documented in the COA when used in accordance with the recommendations in the product sheet and within the stated shelf life of the product.

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