

## Introduction

GF20 MSC Xeno-Free SFM is a medium for Human Mesenchymal Stem Cells (hMSCs), which contains no serum or animal origin components. It can be used for primary culture and subsequent amplification and passage culture of mesenchymal stem cells from the tissues such as human bone marrow, umbilical cord and fat, and maintain their trilineage differentiation potential into mesoderm. This product has superior proliferation promoting ability and short doubling time, which can save precious primary cells and experiment time for you.

## Package Information

Component	C0128
M200 Serum-Free Medium*	490 mL
F20 MSC Supplement	10 mL

\*Type A represents "with phenol red", and Type B represents "no phenol red".

## Storage

M200 Serum-Free Medium at 2°C~-8°C and protect from light;  
F20 MSC Supplement at 2~ 8°C and protect from light.

## Specifications

Classification	Serum-free
Sodium Glutamate	Containing glutamine
Antibiotics	Antibiotics-free
HEPES Buffer	HEPES-free
Sodium Bicarbonate Buffer	Containing sodium bicarbonate
Endotoxin Level	<0.24 EU/mL

## Protocol

### Medium Preparation:

The following operations shall be in an aseptic condition:

1. It is recommended to thaw the F20 MSC Supplement at room temperature (15°C to 25°C) or thaw it overnight in a refrigerator (2°C to 8°C). If necessary, the Supplement can be subpackaged in sterile conditions and stored at -80°C to -20°C.
2. Thaw 10 mL of F20 MSC Supplement completely in sterile conditions, and add it into 490 mL of M200 Serum-Free Medium to prepare a complete medium with a total volume of 500 mL.

**Note:** If necessary, the user can prepare a required dosage in proportion, or add antibiotics by oneself, for example, add penicillin/streptomycin into the complete medium at a dilution ratio of 1:100.

### Cell Culture:

1. Count the Human Mesenchymal Stem Cells (hMSCs) harvested from resuscitation or passage.
2. Add the medium to a desired cell concentration and inoculate the cells into a culture bottle (the generally recommended cell inoculation density is 0.4-0.8×10<sup>4</sup> cells/cm<sup>2</sup>) for culture.
3. Culture Conditions: 5% CO<sub>2</sub>, and 37°C;
4. Change the medium every 2-3 days according to the cell growth, and the cells can be digested when they are 80%-90% confluent;
5. Cell Dissociation Method: Pour out the medium in the culture bottle, and clean the cells stuck to the wall twice with PBS or normal saline; it is recommended to spread 0.05% trypsin or a trypsin substitute (e.g., Biosci™ Trypsin Solution) over the bottom of the culture bottle; incubate the cells in an incubator at 37°C for 1-3 min, add the complete medium with a dosage of more than 5 times of that of the trypsin to terminate digestion, transfer the cell suspension into a centrifugal tube, and conduct centrifugation at 300× g for 5 min. The precipitate is the required cells.
6. Count the cells, and harvest them directly, or further inoculate and culture them as required.

### Note:

1. If the Supplement in this product cannot be used up at one time, it can be subpackaged and stored in a freezing mode, but it shall not be frozen and thawed repeatedly, and shall be used within the validity of the product. After the complete medium is prepared by mixing, it is recommended to be stored away from light at 2°C to 8°C and used up within two weeks, and attention shall be paid to operate in sterile conditions.
2. As the product has high amplification efficiency, the recommended cell inoculation density is 0.4-0.8×10<sup>4</sup> cells/cm<sup>2</sup>, and the user may determine the cell inoculation density as required and according to the actual situation.
3. Compared with a medium containing serum, this product contains no serum, so the trypsin digestion terminating effect is poor, and excessive trypsin residue will cause damage to cells. Therefore, it is recommended to use 0.05% trypsin or a trypsin substitute for digestion when this product is used for cell culture and passage, thus to reduce the damage to cells.
4. The cell culture effect of this product may vary depending on cell sources, storage conditions, sample quality and operator experience.