ExCell

EIV12023

# ExCell Bio

# OptiVitro®
# MSC Expansion Medium XF

For Research and Manufacturing Use
Not Intended for Diagnostic and Therapeutic Use

## User Manual

Catalog Number ME000-N023

ME000-N023S

## Product Description

OptiVitro® MSC (Mesenchymal Stem Cells) Expansion Medium XF (Xeno-free), is specially optimized for the culture and expansion of human mesenchymal stem cells (hMSCs), under completely serum-free and xeno-free conditions. OptiVitro® MSC Expansion Medium supports the long-term cell culture and expansion of MSCs for multiple passages, while maintaining their various differentiation potentials (i.e.,ability to differentiate into osteocytes, chondrocytes, and adipocytes).

## Contents and Storage

| Product | Catalog No. | Amount | Storage | Shelf Life |
|---|---|---|---|---|
| **MSC Expansion Medium XF** | **ME000-N023** | **1 kit** | — | — |
| MSC XSFM Basal Medium | BA0213 | 500 mL | 2-8°C<br>Protect from Light | 9 months |
| MSC XSFM Supplement | BA0021 | 5 mL | –20°C<br>Protect from Light | 12 months |
| **MSC Expansion Medium XF（Sample）** | **ME000-N023S** | **1 kit** | — | — |
| MSC XSFM Basal Medium | BA0213S | 100 mL | 2-8°C<br>Protect from Light | 9 months |
| MSC XSFM Supplement | BA0021S | 1 mL | –20°C<br>Protect from Light | 12 months |
| **Related Product:**<br>Recombinant Trypsin Digestive Solution RF01 | RF000-N031 | 200 mL | 2-8°C<br>Protect from Light | 18 months |

## Applications and Restrictions

OptiVitro® MSC Expansion Medium is only for scientific research and commercial production, not for clinical diagnosis and treatment. The safety and efficacy of this product in clinical diagnostic and therapeutic applications have not been established.

To attain the ideal culture effect, OptiVitro® MSC Expansion Medium can be used directly, or add additional cell growth factors or hormones according to the cell type or research needs.

The experiment results may vary depending on the difference in the mesenchymal stem cell/precursor cell of the human donor cell lines

OptiVitro® MSC Expansion Medium does not contain serum, heterologous components, and antibiotics. Extra supplements can be added if necessary.

OptiVitro® MSC Expansion Medium needs to be used within its expiry date.

## Instructions for Use

### Stability and Storage

OptiVitro® MSC XSFM Basal Medium, should be stored at 2-8℃ away from light (avoid direct sunlight or ultraviolet lamps, fluorescent lamps will not be affected in daily use, please store in a light-proof refrigerator for long-term storage). The product can maintain stable performance within its expiry date.

OptiVitro® MSC XSFM Supplement, should be stored at -20°C. The product can maintain stable performance within its expiry date.

- Thaw OptiVitro® MSC XSFM Supplement at room temperature, shake well and let stand for 5 minutes to dissolve the solution evenly before using or sub packaging, repeated freezing and thawing no more than 3 times, the sub packaged reagents can be stored at -20℃ for 3 months, or temporarily stored at 2-8°C and use up within 1 month.

### Materials and Reagents

1. **Equipment and Materials (BYO)**

Human Umbilical Cord-derived Mesenchymal Stem Cells (hUC-MSCs); Recombinant Trypsin Digestive Solution RF01 (ExCell Bio, RF000-N031); PBS; T-175 cell culture flask; 15 mL, 50 mL centrifuge tube; pipette; electronic pipette; Carbon dioxide incubator; centrifuge; cell counter or blood counting chamber; inverted microscope; water bath kettle, etc..

2. **Prepare Complete Medium**

(1) For 500 mL complete medium, aseptically add 5mL of OptiVitro® MSC XSFM Supplement (BA0021) into 500 mL of OptiVitro® MSC XSFM Basal Medium (BA0213). After mixing well, it can be used as the complete medium to culture MSCs.

(2) After the complete medium is prepared, store it at 2-8℃, avoid direct sunlight and ultraviolet light, and use it up within 2 weeks.

### Culture of MSCs

1. Pre-warm an appropriate amount of complete medium to 37℃ before use (35-53 mL per T-175 flask).

2. Recover or subculture to collect UC-MSCs, resuspend the cells with complete medium according to the cell number or cell density, evaluate the seeding density as needed, and seed MSCs in OptiVitro® MSC Expansion Medium.

**Note 1：** As for the cryopreserved cells cultured in other culture systems, when using OptiVitro® MSC Expansion Medium for the first time, it is recommended to use a 1:1 mixed medium, that is, the OptiVitro® MSC Expansion Medium: the medium used before cryopreservation = 1:1.

**Note 2：** For different sizes of culture flask, the recommended seeding density is low density 3000-5000/$cm^2$ for 72-96h subculture; or high density 8000-10000/$cm^2$ for 48-72h subculture; 0.2-0.3mL medium for each $cm^2$ (35-53mL complete medium per T-175 culture flask, re-feed the culture every 48-72h).

3. Culture the cells with OptiVitro® MSC Expansion Medium in the incubator at 37°C in a humidified atmosphere of 5% $CO_2$. Replace the spent medium every 2-3 days with the pre-warm complete medium.

**Note：** When changing the medium, add the fresh medium to the bottom of the culture flask, and avoid blowing directly on the surface of the cell culture which might damage the cells.

4. When the cells expand to reach 80-90% confluence, propagate the cells, and do not let the cells expansion exceed 90% confluence or completely cover the bottom of the bottle.

## Subculture of MSCs

According to the cell number, the pre-warm appropriate volume of the complete medium at 37℃. Each culture flask is expected to require 45-63 mL of complete medium, 20 mL of PBS buffer, and 10 mL of digestive solution.

1. Washing：Remove the spent medium from the culture flask and discard, wash the cell surface with 10 mL of PBS for each T-175 culture flask, and remove and discard.

2. Digestion：Add 10mL of **Recombinant Trypsin Digestive Solution RF01**, and tilt the flask in all directions to evenly distribute. Digest at room temperature for 2-6 minutes, shake and pat the flask, and check the flask under the microscope. When more than 80% of the cells detached, add an equal volume of complete medium or PBS solution to dilute the digestive solution. Pipetting to disperse the cells into single cells, and count the cells number.

**Note：** When using the culture bottle, tap the bottle gently after digestion to detach the cell. If the digestion is not complete, continue to digest for 1-2 minutes.

3. Collection：Centrifuge the tubes at 300g for 5 minutes to collect the cell pellet.

4. Washing: Add 10mL PBS solution to resuspend the cells, centrifuge at 300g for 5min, discard the supernatant, and collect the cell pellet. **(Recommended Step 4: can avoid the impact of residual digestive solution after cell digestion on cell adherence in the serum-free system).**

**Note 1：** For the primary MSCs isolated by explant method, when they are passaged for the first time (P0 to P1), the cell attachment in the serum-free system is easily affected by the digestive solution, and the cells need to

be washed with PBS after digestion.

**Note 2：** Do not leave the cells in the centrifuge tube for a long time. If the operation time is too long (placed in the culture medium for more than 15 minutes), some cells will adhere to the wall of the tube and cause loss.

5. Seeding：Resuspend the cells with complete medium, inoculate the cell suspension in multiple T-175 culture flasks according to the cell number of 1.40-1.75×$10^6$ per flask, and add complete medium to 35-53 mL per flask.

6. Culture：Culture the cells with OptiVitro® MSC Expansion Medium in the incubator at 37°C in a humidified atmosphere of 5% $CO_2$.

7. Cryopreservation： After step 4, slowly add cell cryopreservation solution and gently mix to resuspend the cells. (**Note:** take the cryopreservation solution immediately and put it back to 4℃ in time), add the suspension to the cryovials and mark it, then place the cryovials in the programmed cooling box (ExCell, CS041-0001) at -80°C overnight, and transfer to liquid nitrogen for long-term storage after 24 hours.

## Safety Information

This product contains human serum albumin, the materials used are clinically applicable raw materials approved by the state, with clear sources, batch numbers and quality reports. The human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HBsAg. However, this medium should still be treated as a potential source of infection. When using it, strictly follow the safety experiment manual, and wear protective equipment to avoid direct contact. The short- and long-term effects of overexposure to this medium are unknown.