

# TransStem<sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Medium

Please read the datasheet carefully prior to use.

Cat. No. MM101

Storage: at the proper storage temperature for one year

## Description

TransStem<sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Medium is a complete medium suitable for isolation, expansion and recovery of mesenchymal stem cells (Human Mesenchymal Stromal/Stem Cells, hMSCs) derived from human pluripotent stem cells, umbilical cord, bone marrow, and adipose, etc. under serum-free, animal-derived component-free conditions. This product has good stability, enabling stable passages of hMSCs to 15 generations. The hMSCs cultured with this product have high ability to differentiate into osteogenic, cartilage, and adipocyte lineages and strong immune regulation ability.

## Kit Contents

Component	MM101-01	Storage
TransStem <sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Basal Medium	500 ml	2-8°C
TransStem <sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Supplement	5×5 ml	At -20°C in the dark, avoid repeated freeze-thawing

## Procedures

### Materials required but not included

Product Name	Volume
PBS (1×)	X µl (200 ng~2 µg)
Fibronectin Human, Plasma	2 µl
TrypLE <sup>™</sup> Express Enzyme	1 µl
TransStem <sup>®</sup> Chemically Defined Xeno-free Cell Cryopreservation Medium	4 µl
TransStem <sup>®</sup> Human Umbilical Cord Mesenchymal Stromal Cells	To 20 µl

### 1. Preparation of complete medium

Add thawed 5 ml TransStem<sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Supplement to 95 ml TransStem<sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Basal Medium. Mix well.

### 2. Petri dish coating

Dilute Fibronectin to 10 µg/ml with 1×PBS, and add it to the culture dish to be coated (please refer to the table below for coating amount) Incubate at 37°C for 1 hour or at 2-8°C overnight. To avoid drying of the substrateduring incubation, discard the substrate to inoculate cells when using.

Note: Diluted Fibronectin cannot be stored for a long time. Please dilute before use.

Cell culture plate (dish)	Area	Coating amount per well
24-well	2 cm <sup>2</sup>	0.25 ml
12-well	4 cm <sup>2</sup>	0.5 ml
6-well	10 cm <sup>2</sup>	1 ml
35 mm	10 cm <sup>2</sup>	1 ml
60 mm	20 cm <sup>2</sup>	2 ml
100 mm	60 cm <sup>2</sup>	6 ml



### 3. Recovery of Human Mesenchymal Stromal/ Stem Cells

- (1) Add 5-10 ml of complete medium equilibrated to room temperature in a 15 ml centrifuge tube.
- (2) Remove mesenchymal stem cells from liquid nitrogen (*TransStem*<sup>®</sup> Human Umbilical Cord Mesenchymal Stromal Cells are recommended, catalog number: MC401-01 ), and quickly thaw by shaking in a 37°C water bath.
- (3) Pipet the cell suspension in the cryovial to the pre-prepared complete medium. Mix gently, and centrifuge at 300×g for 5 minutes. Discard the supernatant. The cells are resuspended by adding an appropriate amount of complete medium equilibrated to room temperature and seeded into a petri dish pre-coated with Fibronectin. The recommended seeding density is 1-2×10<sup>4</sup> viable cells/cm<sup>2</sup>.
- (4) Incubate in a CO<sub>2</sub> incubator and change the medium after 24 hours. Change the medium every other day.

### 4. Passaging expansion of Human Mesenchymal Stromal/ Stem Cells

- (1) Observe under the microscope, and passage the cells when the confluence reaches 80%-90%.
- (2) Aspirate and discard the spent culture medium. Wash once with 1×PBS. Add 500 µl-1 ml of pre-warmed (at 37°C ) *TrypLE*<sup>™</sup> Express Enzyme to each well of the 6-well plate. Digest at 37°C for 5 minutes, and wait until the cells are completely detached.
- (3) Add 2 ml of complete medium equilibrated to room temperature and pipette gently to form a single-cell suspension.
- (4) Centrifuge at 300×g for 5 minutes, and discard the supernatant. Add an appropriate amount of complete medium equilibrated to room temperature to resuspend the cells, and count the cells. It is recommended to inoculate the cells at a density of 5×10<sup>3</sup>-2×10<sup>4</sup> cells/cm<sup>2</sup> to pre-coated Fibronectin cells in a petri dish.
- (5) Incubate in a CO<sub>2</sub> incubator and change the medium after 24 hours. Change the medium every other day.

### 5. Cryopreservation of Human Mesenchymal Stromal/ Stem Cells

*TransStem*<sup>®</sup> Chemically Defined Xeno-free Cell Cryopreservation Medium (Cat. No. MC101-01) is recommended for cryopreservation of cells.

- (1) Aspirate and discard the spent culture medium. Wash once with 1×PBS. Add 500 µl-1 ml of pre-warmed (at 37°C ) *TrypLE*<sup>™</sup> Express Enzyme to each well of the 6-well plate. Digest at 37°C for 5 minutes, and wait until the cells are completely detached.
- (2) Add 2 ml of complete medium equilibrated to room temperature and pipette gently to form a single-cell suspension.
- (3) Centrifuge at 300×g for 5 minutes, discard the supernatant and add an appropriate amount of 2-8°C pre-cooled cell cryopreservation medium to resuspend the cells. Cryopreservation at a density of 2-4×10<sup>6</sup> cells/ml is recommended.
- (4) Transfer the cell suspension to a cryopreservation tube. Place it in a programmed cooling box in a -80°C refrigerator overnight, and transfer to a liquid nitrogen tank the next day.

#### Notes

- Please thaw *TransStem*<sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Supplement at 2-8°C or room temperature (22-25°C, no more than 4 hours).
- *TransStem*<sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Supplement cannot be subject to repeated freeze-thawing. Please divide it into a single-use amount and store it at -20°C before use.
- For *TransStem*<sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Medium with or without *TransStem*<sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Supplement, precipitation is a normal phenomenon, which does not affect the performance of the product. If necessary, complete media can be filtered using a 0.22 µm filter which is not recommended for *TransStem*<sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Supplement.
- The prepared complete medium should be stored at 2-8°C away from light, and used within two weeks. Do not re-freeze.
- Before using the complete medium, take it out of the refrigerator and equilibrate to room temperature. Please do not heat it in a 37°C water bath.

FOR RESEARCH USE ONLY

