

StemGro® Mesenchymal Stem Cell (MSC) Expansion Medium

Cat.No.	Product	Amount	Shelf life	Type	storage	Transportation
T310JV	StemGro® Mesenchymal Stem Cell (MSC) Expansion Medium	100 mL	12 months	Liquid	-30 ~ -5 °C	Dry ice
T310KJ	StemGro® Mesenchymal Stem Cell (MSC) Expansion Medium	500 mL	12 months	Liquid	-30 ~ -5 °C	Dry ice



1.Introductions

StemGro® Mesenchymal Stem Cell (MSC) Expansion Medium is a Xeno-free medium specifically designed for human stem cells (including umbilical cord mesenchymal, bone marrow mesenchymal, adipose mesenchymal and umbilical cord blood mesenchymal stem cells). When cell adherence reaches 75 ~ 85% during stem cells isolation, this medium can be used for cell passage cultivation. Compared to traditional media which require the additional of animal serum, StemGro® Mesenchymal Stem Cell (MSC) Expansion Medium has the ability to sustain undifferentiated growth of mesenchymal stem cells and maintain the morphological characteristics and karyotypes of cells well. Furthermore, it can sustain the high proliferation rate of cells as well as differentiating potential of mesenchymal stem cells. This medium is able to eliminate the possibility of mixing of Xeno (components from animal species other than human) during culturing.

The product is prepared using water-for-injection.

This product is documented as Class I medical devices under NMPA. It can be used for scientific research and in vitro diagnosis.

2. Quality management system

The product is manufactured in CGMP standard workshop in Basalmedia Shanghai.

Basalmedia Shanghai Biotechnology Co.Ltd has obtained ISO9001 :2015 , ISO13485 :2016 quality system certification.

3. Product parameters

The product is sterilized by filtration.

Appearance: Transparent liquid with red color

Endotoxin: ≤3 EU/mL

Osmotic pressure: 270 ~ 340 mOsm/kg·H₂O

pH value: 7.0 ~ 7.4

Storage: -30 ~ -5 °C

Transprotation: Dry ice

Application: for research and production only.

4.Use guide

Please wear appropriate safety gloves, lab clothes and goggles when using. The product should not be used on the human body.

The environment in which the cells contact directly must be sterile, and the reagents used in cells culture must be sterile. Please conduct cell experiments in a sterile environment. Before moving any vessels or tools into a sterile environment, remove the outer packaging at the entrance and disinfect with alcohol swab.

Note: Do not use the product when it has completely melted upon receipt, or when it has passed its term of validity.

Note: At a certain temperature during transportation or storage (validity period), if there is a decrease in clarity, this is the precipitation of some protein components, which can be mixed before use, The effect of cell culture will not be affected.

5.Prepare medium

StemGro® Mesenchymal Stem Cells (MSC) Expansion medium is frozen before use. Please thaw gently at 2 ~ 8 °C and mix by gentle swirling to ensure the medium uniform. Do not thaw the medium in the environment ≥ 37 °C! Use immediately after thawing. Thawed and unopened StemGro® mesenchymal stem cells (MSC) expansion medium can be stored at 2 to 8 °C for 1 month

6. Culture conditions

StemGro® Mesenchymal Stem Cells (MSC) Expansion medium is a complete medium with no need to add other components. antibiotics can be used if desired, Gentamicin Reagent Solution (5 µg/mL final concentration) to prevent contamination of isolated stem cells.

Cell lines: Umbilical cord mesenchymal, bone marrow mesenchymal, adipose mesenchymal and umbilical cord blood mesenchymal stem cells

Culture type: Adherent

Culture vessels and equipment: Culture plate, flask and CO₂ incubator.

Incubator atmosphere: humidified atmosphere of 5% CO₂ in air, protect from light.

Set the temperature and air conditions of the cell culture incubator before the experiment.

7. Recover cryopreserved cells

1. Rapidly thaw (<1 minute) frozen vial of cells in a 37°C water bath. Remove vial from water bath just before the last trace of ice has melted.;
2. Rinse a pipette tip with complete medium and very gently transfer the cells from the cryovial to a pre-rinsed 15-mL conical tube.;
3. Add the pre-warmed StemGro® mesenchymal stem cells (MSC) Expansion medium slowly at a rate of drop per 2 seconds, Mix by gentle swirling after each drop;
4. Centrifuge at room temperature with 100 ~ 200 × g for 5 min, and then aspirate the supernatant;
5. Resuspend the cells by adding the medium with the minimum volume, count the cells with a cell counter, and calculate the living cell density;
6. Rinse the T75 flask with the medium, add appropriate amount of pre-warmed complete StemGro® mesenchymal stem cells (MSC) Expansion medium, and then add the cell resuspend to ensure the density of live cells plated in the culture flask is about 5 × 10³ cells /cm²;
7. Culture in the incubator;
8. Refresh the medium every 2 or 3 days, pre-warm the medium before use.

8. Cell passage

Cells passage using StemGro® mesenchymal stem cells (MSC) expansion medium

1. Passage when the cells are 70 ~ 80 % confluent;
2. Pre-warm the Trpzyme® Recombinant Trypsin Solution (without Animal-derived component, Cat.No. S342JV) and StemGro® mesenchymal stem cells (MSC) expansion medium before use;
3. Aspirate the medium in the culture flask and Rinsed the

cells with DPBS (without Ca²⁺ and mg²⁺), then remove the DPBS.

4. Add 3 ~ 5 mL pre-warmed Trpzyme® Recombinant Trypsin Solution, Make sure the solution covers all surfaces of the culture. Culture for 5 to 10 minutes with the recommended cell culture conditions;
 5. Use an inverted microscope to observe the cell culture flask to make sure the cells separate completely;
 6. Add pre-warmed DPBS (containing Ca²⁺ and mg²⁺) to each flask to ensure that the DPBS completely covered the culture surface. Transfer the cell suspension into a 15 mL sterile centrifuge tube. Rinse the flask again with 5 mL of DPBS (containing Ca²⁺ and mg²⁺), then transfer into the centrifuge tube;
 7. Centrifuge at 100 ~ 200 × g at room temperature for 5 minutes, then aspirate the supernatant with cautious;
 8. Resuspend the cells using the minimum volume of StemGro® mesenchymal stem cells (MSC) expansion medium for live cell counting;
 9. Add 15 mL pre-warmed StemGro® mesenchymal stem cells (MSC) expansion medium to flask;
 10. Add enough cell suspension to each flask to provide ≥ 5 × 10³ cells/cm² (i.e. 3.75 × 10⁵ cells per T-75 flask). Mix or swirl the cell suspension to ensure even distribution;
 11. Place the culture flask(s) in the incubator at 36°C to 38°C with a humidified atmosphere of 4~6% CO₂ .;
 12. Refresh the culture medium every 2 or 3 days;
- Note: It is recommended that passage mesenchymal stem cells less than 5 generations.

9. Cryopreserve cells

Before the Cryopreservation, prepare a sufficient amount of cells which are 80%~90% confluent:

1. Prepare cryopreservation medium (46.25 % fresh CD medium+46.25 % conditioned medium+7.5 %DMSO) and store at 2~8 °C (less than 24 hours). Avoid light. CD-Freezer® chemically defined cryopreservation medium (S919JV, 7.5%DMSO included) is recommended.
2. Determine the number of cells and calculate the required cryopreservation medium volume V (the recommended

- cryopreservation density is $1-5 \times 10^6$ cells /mL);
- Aspirate the medium in the culture flask, and then rinse the cells with 5 mL of DPBS without Ca^{2+} and Mg^{2+} . After rinsing, remove the DPBS;
 - Add 3 mL Trpzyme® Recombinant Trypsin Solution (add 1 mL solution for T 25 flask) ;
 - Place at room temperature for 2 ~ 5 minutes, gently tap the bottle wall to help the cells disassemble; After the cells are removed from the flask wall, add the cryopreservation medium prepared in Step 1 of V mL immediately. Tilt and shake the flask to mix the solution, and fully contact all corners of the flask inner wall;
 - Divide the cell resuspension into cell cryopreservation tubes (usually 1.5ml per tube) according to subsequent use requirements.;
 - Mark on the cryovial (cell name, cryopreserve time and operator e.g.) ;
 - Programmed thermostat can be used to control the temperature (the standard cryopreservation cooling rate is $-1 \sim -2$ °C/ min). When the temperature is below -25 °C, the temperature deceleration rate can be increased to -5 °C ~ -10 °C/ min; At -100 °C, it can be immersed in liquid nitrogen rapidly;
 - Artificial cooling method can also be used: put the cell cryopreservation tube into the Nalgene containing isopropyl alcohol, put it in the -20 °C refrigerator for 2 hours, then put it in the -80 °C refrigerator overnight, and finally take out the cryopreservation tube separately and move it into the liquid nitrogen container;

10.Related products

Cat.No.	Product	Amount	Storage	Transportation
T311JV	StemGro® Mesenchymal Stem Cell (MSC) Expansion Medium, no phenol red	100 mL	$-30 \sim -5$ °C	Dry ice
T311KJ	StemGro® Mesenchymal Stem Cell (MSC) Expansion Medium, no phenol red	500 mL	$-30 \sim -5$ °C	Dry ice
S342JV	Trpzyme® Recombinant Trypsin Solution, no phenol red	100 mL	$-30 \sim -5$ °C	Dry ice
S342KJ	Trpzyme® Recombinant Trypsin Solution, no phenol red	500 mL	$-30 \sim -5$ °C	Dry ice
S919JV	CD-Freezer® chemically defined cryopreservation medium	100 mL	$2 \sim 8$ °C	Blue ice
B210KJ	Dulbecco's DPBS, without Ca^{2+} , Mg^{2+} , Phenol red	500 mL	$2 \sim 30$ °C	RT
B310KJ	phosphate buffered saline (PBS), pH7.2	500 mL	$2 \sim 30$ °C	RT
B320KJ	phosphate buffered saline (PBS), pH7.4	500 mL	$2 \sim 30$ °C	RT