

USER MANUAL

Maintenance of Cellartis® hES-MP 002.5

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General information

Catalogue number: Y10090/MPC-301-VIAL 1 frozen vial of Cellartis® hES-MP 002.5

Cellartis® hES-MP 002.5 are human mesenchymal progenitor cells derived from a human embryonic stem cell line. The cells have been differentiated to mesenchymal progenitor cells in vitro and subsequently been dissociated to a single cell suspension and frozen in vials.

Cellartis® hES-MP™002.5 are shipped on dry ice or in a dry shipper and should be handled according to “Unpacking and Handling” upon arrival.

The Cellartis® hES-MP 002.5 are frozen in passage 5 and can be passaged for at least 10 passages. It is recommend to use culture flasks of 75 or 150 cm² when culturing the cells.

We recommend that this product is handled only by persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good cell culture practice.

Additional Material Required

Product	Suggested Manufacturer	Catalogue number
basic Fibroblast Growth Factor (bFGF)	Peptotech	100-18B
Dulbecco's Modified Eagle Medium (DMEM)	Gibco/Life Technologies	31966-021
Foetal Bovine Serum (FBS)	Gibco/Life Technologies	16140
Gelatine	Sigma-Aldrich	G1890
PBS Dulbecco's w/o Ca ²⁺ & Mg ²⁺ (D-PBS (-/-))	Gibco/Life Technologies	14190
Penicillin/Streptomycin (PEST)	Gibco/Life Technologies	15140-122
TrypLE Select™	Gibco/Life Technologies	12563-011

Other Equipment Needed

General cell culture equipment used in cell culture laboratory.

Product Quality

Takara Bio Europe AB recommends the use of media and reagents according to this manual. Takara Bio Europe AB cannot give technical feedback on customer cultures unless the below culture instructions have been followed.

Methods

NOTE! Always work under aseptic conditions.

Unpacking of Cellartis® hES-MP 002.5

NOTE! For your protection: Wear a protective face mask and protective gloves. Use forceps when handling a frozen vial. Never hold the vial in your hand as the cryo vial may explode due to rapid temperature changes.

NOTE! When transferring the cells from the transport vessel to long time storage, **immediate** transfer is essential since variations in temperature may have an adverse effect on cell survival and quality.

1. Unpack Cellartis® hES-MP 002.5 as soon as possible after arrival.
2. Check all materials for leakage or breakage.
3. Using forceps immediately place the vial in liquid nitrogen at ≤ -150°C.

Storage and Handling of Cellartis® hES-MP 002.5

Cellartis® hES-MP 002.5 should be stored at ≤ -150°C. Under recommended storage conditions the cells can be stored for up to one year from date of receipt.

Keep thawed cells at $+37\pm 1^{\circ}\text{C}$, 5 % CO_2 , and >90 % humidity. Once thawed, the cells can be maintained in culture for at least 30 passages under the recommended conditions.

Cellartis® hES-MP 002.5 Medium Preparation

Cellartis® hES-MP 002.5 Basal Medium

1. Decontaminate the external surface of all supplements and medium bottle with appropriate disinfectant.
2. Add 10 % FBS and 1 % PEST to DMEM to achieve Cellartis® hES-MP 002.5 Basal Medium.
3. Cellartis® hES-MP 002.5 Basal Medium should be stored at $+2-8^{\circ}\text{C}$ and expires one month after the date of preparation.
4. Before use, warm to room temperature (RT, $+15-25^{\circ}\text{C}$). Discard any leftover warm Cellartis® hES-MP 002.5 Basal Medium.

Cellartis® hES-MP 002.5 Culture Medium

1. Prepare Cellartis® hES-MP 002.5 Culture Medium immediately before use.
2. Decontaminate the external surface of all supplements and medium bottle with appropriate disinfectant.
3. Add bFGF to a final concentration of 4 ng/ml to Cellartis® hES-MP 002.5 Basal Medium to achieve Cellartis® hES-MP 002.5 Culture Medium.
4. Before use, warm to RT. Discard any leftover warm Cellartis® hES-MP 002.5 Culture Medium.

Coating of Cell Culture Units

1. Coat the cell culture units with 0.1 % autoclaved gelatine solution, 0.2-0.3 mL/cm². Make sure the entire surface is covered.
2. Incubate at RT for 0.5-24 hour.
3. Remove the gelatine solution from the cell culture units by aspiration, just before use.

Thawing of Cellartis® hES-MP 002.5

It is recommended to seed cells from one vial of Cellartis® hES-MP 002.5 in one 75 cm² cell culture flask.

Preparation

1. Prepare appropriate volume of Cellartis® hES-MP 002.5 Basal Medium and Culture Medium. Warm to RT.
2. Coat the appropriate number of cell culture units according to "Coating of cell culture units".

NOTE! For your protection: Wear a protective face mask and protective gloves. Use forceps when handling a frozen vial. Never hold the vial in your hand as the cryo vial may explode due to rapid temperature changes.

Thawing Cells

1. Transfer 10 mL Cellartis® hES-MP 002.5 Basal Medium to a centrifuge tube.
2. Transfer, as quickly as possible, the frozen vial from liquid nitrogen to a $+37\pm 1^{\circ}\text{C}$ water bath.
3. Thaw the cells without swirling the vial. Take the vial out of the water bath as soon as the thawing is completed.
4. Wipe the vial with appropriate disinfectant and place in a safety hood.
5. As soon as possible, transfer the cell suspension to the centrifuge tube with basal medium.
6. Centrifuge the tube at 300g at RT, for 5 minutes and remove the supernatant.
7. Discard the supernatant and resuspend the pellet in 10-15 mL Cellartis® hES-MP 002.5 Culture Medium.
8. Seed the cells in the gelatine coated 75 cm² cell culture flask.
9. Transfer the cell culture unit to an incubator with $+37\pm 1^{\circ}\text{C}$, 5 % CO_2 , and >90 % humidity.
10. Evaluate the Cellartis® hES-MP 002.5 cells under a microscope the day after thawing and change medium if a lot of debris is present.

Medium Change of Cellartis® hES-MP 002.5

Change medium every 2nd to 3rd day.

1. Prepare appropriate volume of Cellartis® hES-MP 002.5 Culture Medium, 0.13-0.20 mL medium/cm², and warm to RT.
2. Remove the entire volume of medium from the cell culture unit and discard.
3. Add warm Cellartis® hES-MP 002.5 Culture Medium to achieve the final volume.
4. Return the cell culture unit to +37±1°C.

Passage of Cellartis® hES-MP 002.5 cells

Passage the Cellartis® hES-MP 002.5 cells every 3rd-7th day or when the cell confluence is approximately 70-90 %. Seed the Cellartis® hES-MP 002.5 cells at a density of 8-12k cells/cm².

1. Prepare appropriate volume of Cellartis® hES-MP 002.5 Basal Medium and Culture Medium. Warm to RT.
2. Coat the appropriate number of cell culture units according to “Coating of cell culture units”.
3. Remove the entire volume of medium from the cell culture units and discard.
4. Rinse the cell surface by adding D-PBS (-/-), use approximately 0.1 mL/cm². The entire surface should be covered.
5. Remove the D-PBS (-/-) and add TrypLE Select™, use approximately 0.05 mL/cm². The entire surface should be covered.
6. Incubate at +37±1°C for 5 minutes. Check cells under a microscope to make sure the cells have detached from the surface. If not, incubate for 1-3 additional minutes in incubator.
7. Pipette to a single cell suspension and transfer to a tube with Cellartis® hES-MP 002.5 Basal Medium, same volume as the volume of TrypLE Select™ used.
8. Centrifuge the cell suspension at 300g for 5 minutes.
9. Discard the supernatant and re-suspend the pellet in Cellartis® hES-MP 002.5 Culture Medium.
10. Count the cells and seed them in accordance with recommendations stated above to gelatine coated cell culture flasks. Add Cellartis® hES-MP 002.5 Culture Medium to final volume of 0.13-0.20 mL medium/cm².
11. Place the cell culture flask/flasks in incubator with +37±1°C, 5 % CO₂, and >90 % humidity.

For technical support email: tech-cellartis@takara-clontech.eu

Authorised uses

Except as otherwise agreed in writing, the purchase of goods only conveys to you the non-transferable right for only you to use the quantity of goods and components of goods purchased in compliance with the applicable intended use statement. Unless otherwise authorized, no right to resell the goods, or any portion of them, is conveyed hereunder.

The goods are intended for research use only and are not to be used for any other purposes including, but not limited to: unauthorized commercial purposes, *in vitro* diagnostic purposes, *ex vivo* or *in vivo* therapeutic purposes, investigational use, in foods, drugs, devices or cosmetics of any kind, or for consumption by or use in connection with or administration or application to humans or animals.