ExCell

EIV62025E

ExCell Bio

MSC Serum-free Medium MH02

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number MH000-N022

MH000-N021

MH000-N021S

PRODUCT DESCRIPTION

MSC (Mesenchymal Stem Cells) Serum-free Medium MH02 is a serum-free, xeno-free medium that specially optimized for the primary cell isolation and expansion of human mesenchymal stem cells (hMSCs), under complete serum-free, phenol red-free and xeno-free conditions. MSC Serum-free Medium MH02 is recommended to be used with 2-5% hPL. The complete medium with 2-5% hPL supports the isolation of primary MSCs by explant and digestion methods, and expansion of MSCs for multiple passages, while maintaining the stability of surface markers.

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Product Name	Cat.#	Specification	Storage	Transportation	Shelf Life
MSC Serum-free Medium MH02	**MH000-N022**	**1000 mL kit**	-	-	-
MSC Serum-free Basal Medium MH01	BA0302	1000 mL	2-8°C Protect from light	< 25°C Protect from light	12 months
MSC Serum-free Medium Supplement MH02	BA0322	1 mL	-20°C Protect from light	< 0°C Protect from light	12 months
MSC Serum-free Medium MH02	**MH000-N011**	**500 mL kit**	-	-	-
MSC Serum-free Basal Medium MH01	BA0301	500 mL	2-8°C Protect from light	< 25°C Protect from light	12 months
MSC Serum-free Medium Supplement MH02	BA0321	0.5 mL	-20°C Protect from light	< 0°C Protect from light	12 months
MSC Serum-free Medium MH02(Sample)	**MH000-N021S**	**100 mL kit**	-	-	-
MSC Serum-free Basal Medium MH01 (Sample)	BA0301S	100 mL	2-8°C Protect from light	< 25°C Protect from light	12 months
MSC Serum-free Medium Supplement MH02(Sample)	BA0321S	0.1 mL	-20°C Protect from light	< 0°C Protect from light	12 months

HANDLING RECOMMENDATIONS

MSC Serum-free Basal Medium MH01

1. Please make sure to store the cell culture medium in a light-protected environment, avoid fluorescent lamps or other lamplight exposure, and better to use colored packaging bags in the refrigerator or warehouse.
2. During the transportation of the product, it should be kept away from light. This is to prevent the product from being affected by the irradiation of fluorescent lamps or other light sources, which may lead to discoloration.
3. During the transportation of the product to the clean area, it is essential to carry out a cleaning process. The cleaning method may involve disinfectant wiping, and not utilize UV irradiation.

Note: *When passing through transfer windows equipped with UV lamp, remember to proactively turn off the UV lamp inside the transfer window.*

MSC Serum-free Medium Supplement MH02

1. The product must be stored at -20°C. Please make sure to store the cell culture medium in a light-protected environment, avoid fluorescent lamps or other lamplight exposure, and better to use colored packaging bags in the refrigerator or warehouse.
2. Shipping Instructions: Ship on dry ice. Upon receipt, inspect the package for presence of dry ice. If compromised conditions are observed (e.g., no dry ice remaining), contact the supplier immediately.
3. Prior to use, thaw the supplement at room temperature, gently invert the bottle 5-10 times to mix, followed by 5 minutes of standing to ensure homogeneous dissolution before use or aliquoting. Do not exceed 3 freeze-thaw cycles. Aliquoted reagents may be stored at -20°C for up to 3 months, or temporarily at 2-8°C for use within 2 weeks.

EXPERIMENTAL MATERIALS AND REAGENTS

Cells	Human Umbilical Cord-derived Mesenchymal Stem Cells (hUC-MSCs)
Reagents and consumables	Human Platelet Lysate (hPL), OptiVitro® Recombinant Trypsin Digestive Solution RF01 (ExCell Bio, RF000-N031), DPBS, T-175 cell culture flask, 15/50 mL centrifuge tube, pipette, electronic pipette
Instruments	Carbon dioxide incubator, centrifuge, cell counter or blood counting chamber, inverted microscope, water bath kettle etc.

INSTRUCTION FOR USE

Preparation of Complete Medium

1. For 500 mL complete medium, aseptically add 0.5 mL of MSC Serum-free Medium Supplement MH02 into 500 mL of MSC Serum-free Basal Medium MH01, then add 10 mL human platelet lysate. After mixing well, it can be used to culture MSCs.

   Note:*If this medium is used for the primary extraction of mesenchymal stem cells, it is recommended to add 5% human platelet lysate. For example, to prepare 500 mL of MSC serum-free amplification medium, add 0.5 mL of MSC serum-free medium to the 500 mL of MSC serum-free basic medium (MH01). Then, add 25 mL of human platelet lysate, mix thoroughly, and use the prepared medium.*
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 (25 mL per T-175 flask).
2. Collect hUC-MSCs, resuspend in complete medium, and adjust the seeding density. Use low density (4000 cells/cm²) for 96 hours of subculture or high density (8000 cells/cm²) for 72 hours.
3. Culture MSCs at 37℃ with 5% CO_2. Subculture when cells reach 80-90% confluence.
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

1. Pre-warm required complete medium, DPBS buffer, digestive solution.
2. Washing: Remove the spent medium from the culture flask and discard, wash the cell surface with 10 mL of DPBS for each T-175 culture flask, remove and discard.
3. Digestion: Add 6 mL of OptiVitro® Recombinant Trypsin Digestive Solution RF01 to the flask and tilt the flask in all directions to evenly distribute digestive solution. Incubate the flask at 37℃ for 3-5 minutes, shake and pat the flask, and observe the cells under the microscope. When more than 80% of the cells are detached, add an equal volume of complete medium or DPBS solution to the flask. Disperse the cells into single cells by pipetting.

   Note:*If the digestion is not complete, continue to digest for 1-2 minutes. If more than 80% of the cells have fallen off, delayed operation may cause the cells to clump and fail to blow apart. If the cell confluence is too high, it is possible that the cells are detached in sheets. In this case please pipet more times to disperse the cells into single cells, or resuspend cells in a smaller volume after centrifuging in order to disperse the cells into single cells.*
4. Collection: Centrifuge the tubes at 300 g for 5 min to collect the cell pellet.
5. Washing: Add 10mL DPBS solution to resuspend the cells, centrifuge at 300 g for 5 min, discard the supernatant, and collect the cell pellet.

   Note:*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.For the primary MSCs isolated by explant method, when they are passaged for the first time (P0 to P1), the cell attachment in the SFM system is easily affected by the digestive solution, and the cells need to be washed with DPBS after digestion.*

6. Seeding: Resuspend the cells with complete medium, seed the cells in multiple T-175 culture flasks according to the number of 1.40×10^6 cells per flask (or the passage ratio of 1:7), and add 25 mL complete medium to each flask.

   Note:*If the seeding density is too high or the culture time is too long, the cells may grow too dense and cause cell clumping.*

7. Culture: Culture the cells with MSC Serum-free Medium MH02 in the incubator at 37°C in a humidified atmosphere of 5% CO_2.
8. Cryopreservation: After step 5 Washing, slowly add cell cryopreservation solution and gently mix to resuspend the cells. 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.

DISCLAIMER

1. Use the product according to the manual instructions. Deviations from these instructions are at the user's risk, and our company will not be responsible for any resulting product performance deviations.
2. This product is for scientific research and commercial production only and is not intended for clinical diagnosis or treatment. Users assume all risks for unauthorized use, and our company shall not be responsible for any consequences.