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ExCell Bio

MSC Serum-free Medium MH01

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	MH000-N012
Catalog Number	MH000-N011
Catalog Number	MH000-N011S



PRODUCT DESCRIPTION

The MSC Serum-free Medium MH01 is a serum-free, xeno-free medium specifically optimized for the isolation and expansion of human mesenchymal stem cells (hMSCs) under fully serum-free, phenol red-free, and xeno-free conditions. It supports primary MSC isolation using explant and enzymatic digestion methods and enables expansion over multiple passages, preserving the stability of cell surface markers.

SPECIFICATION, STORAGE AND TRANSPORTATION

Product Name	Cat.#	Specification	Storage	Transportation	Shelf Life
MSC Serum-free Medium MH01	MH000-N012	1000 mL kit	-	-	-
MSC Serum-free Basal Medium MH01	BA0302	1000 mL	2-8°C Protect	<25°C	12 months
			from light	Protect from light	
MSC Serum-free Medium	BA0312	24 mL	-20°C	< 0°C	12 months
			Protect		
			from light	Protect from light	
MSC Serum-free Medium MH01	MH000-N011	500 mL kit	-	-	-
MSC Serum-free Basal Medium MH01	BA0301	500 mL	2-8°C	< 25°C Protect from light	12 months
			Protect		
			from light		
MSC Serum-free Medium Supplement MH01	BA0311	12 mL	-20°C	< 0°C	12 months
			Protect		
			from light	Protect from light	
MSC Serum-free Medium MH01	MH000-N011S	100 mL kit	-	-	-
MSC Serum-free Basal Medium MH01			2-8°C		
	BA0301S	100 mL	Protect	< 25°C	12 months
			from light	Protect from light	
MSC Serum-free Medium Supplement MH01	BA0311S	2.4 mL	-20°C	< 0°C	
			Protect		12 months
			from light	Protect from light	
Kelated Product:			2-8°C	10500	
OptiVitro® Recombinant	RF000-N031	200 mL	Protect	< 25°C	18 months
Trypsin Digestive Solution			from light	Protect from light	
RF01					

Web: www.excellbio.com

PERFORMANCE, APPLICATION AND HANDLING

RECOMMENDATIONS

1. Store cell culture medium in a dark environment, ideally in colored packaging to protect it from light exposure.

2. During transport, avoid prolonged exposure to fluorescent or other types of lighting to prevent discoloration.

3. For transport to sterile areas, implement thorough cleaning and sterilization methods, such as disinfectant wiping (avoid UV sterilization).

4. For transfer through UV-sterilized windows, switch off UV lamps in advance.

INSTRUCTION FOR USE

Equipment and Materials

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

Preparation of Complete Medium

1. Thaw the MSC Supplement MH01 at room temperature, shake well, and let it stand for 15 minutes.

Avoid repeated freeze-thaw cycles; aliquots can be stored at -20°C for up to 3 months.

- For 500 mL of complete medium, aseptically add 12 mL of MSC Supplement MH01 to 500 mL of MSC Basal Medium MH01.
 - 3. Store prepared medium at 2-8°C, protected from sunlight and UV light, and use within 2 weeks.

Culture of MSCs

- 1. Pre-warm an appropriate amount of complete medium to 37°C before use (35-53 mL per flask).
- 2. Collect hUC-MSCs, resuspend in complete medium, and adjust the seeding density. Use low density

(3000-5000 cells/cm²) for 72-96 hours of subculture or high density (8000-10000 cells/cm²) for 48-72 hours.

3. Culture MSCs at 37°C with 5% CO₂. Subculture when cells reach 80-90% confluence.

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Subculture of MSCs

1. Pre-warm required complete medium (45-63 mL per T-175 flask), DPBS buffer (20 mL per flask), digestive solution (10 mL per flask).

 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 6-10 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°C 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 $1.40-1.75\times10^6$ cells per flask (or the passage ratio of 1:7), and add 35-53 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 MH01 in the incubator at 37°C in a humidified atmosphere of 5% CO₂.

8. Cryopreservation: After step 4, 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. The product should be used according to the instructions in the manual. If the experimenter fails to operate according to the instructions, our company will not be responsible for any deviation in product performance caused by this.

2. The product is only used for scientific research and commercial production, and is not suitable for clinical diagnosis and treatment. Otherwise, all consequences arising shall be borne by the experimenter, and our company shall not be responsible.