hMSC High-Efficiency Cryopreservation Medium

Product Manual

I. Product Introduction

hMSC High-Efficiency Cryopreservation Medium is a serum-free product specifically formulated for the cryopreservation of mesenchymal stem cells (MSCs). This product is specially designed to significantly reduce cell damage during the freezing process, improve the survival rate after cell recovery, and maintain the multilineage differentiation potential of MSCs for a long period. Additionally, due to the stable quality between batches, it is suitable for the preservation of cells at the research level.

II. Product Information

Table 1: hMSC High-Efficiency Cryopreservation Medium Product Description

Product Information	Amount	Cat.No.	Storage
ncMission® hMSC High Efficiency Cryopreservation Solution	50 ml	SN-06-1310	2℃～8℃

III. Storage Conditions

1. Storage Temperature: 4°C.
2. Shelf Life: 12 months.

IV. Cryopreservation of MSC

1. Label the required number of cryovials based on the desired cell quantity (reference quantity: 1-5×10^6 cells/mL/tube).
2. Select cells in the logarithmic growth phase and collect them into a centrifuge tube using standard methods.
3. Centrifuge to collect the cells (reference centrifuge conditions: 200×g for 5 min).
4. Aspirate the supernatant and slowly add an appropriate volume of hMSC High-Efficiency Cryopreservation Medium to the centrifuge tube. Mix thoroughly to create a cell suspension.
5. Aliquot the cell suspension into pre-labeled cryovials.
6. Gradient cooling procedure:
   - 6.1 Place the cryovials containing the cell suspension into a pre-cooled cooling box (e.g., Nalgene Mr. Frosty, 5100001) at 4°C. Then, place the cooling box into a -80°C freezer. After 24 hours, transfer the vials to a liquid nitrogen tank for long-term storage (temperature not exceeding -135°C).
   - 6.2 Alternatively, use a programmed cooling device (e.g., Thermo CryoMed) to cool the cryovials at a rate of -1°C/min to -100°C (refer to the device's user manual), then immediately transfer the vials to a liquid nitrogen tank for long-term storage (temperature not exceeding -135°C).

V. Resuscitation of MSC

1. Remove the frozen cells from the liquid nitrogen tank and transport them on dry ice to the cell culture area.
2. Immediately place the cryovial into a 37°C water bath, gently shaking while rapidly thawing.
3. Once the cell suspension in the cryovial has thawed, leaving only small ice chunks, remove the cryovial, disinfect it, and transfer it into a biosafety cabinet.
4. Immediately transfer the cell suspension into a 15 mL centrifuge tube. Slowly add 9 mL of pre-warmed cell culture medium, gently shaking to mix. Take 1 mL of pre-warmed cell culture medium to rinse the cryovial, then combine the remaining cells into the same centrifuge tube.
5. Centrifuge to collect the cells (reference centrifuge conditions: 200×g for 5 min), and aspirate the supernatant.
6. Add 1-2 mL of complete medium to resuspend the cells.
7. Seed the cells into the culture vessel at an appropriate seeding density, adding an appropriate volume of pre-warmed fresh complete cell culture medium.
8. Gently mix the cells using a cross-shaking method, then incubate at 37°C, 5% CO2, and saturated humidity in a cell culture incubator.