hMSC Mild Digestion Solution

Product Manual

I. Product Introduction

hMSC Mild Digestion Solution is an animal origin-free, recombinant enzyme specifically used for dissociating human mesenchymal stem cells. It has a gentle digestive effect, making it suitable for subculturing of a various MSC.

II. Product Information

Table 1: hMSC Mild Digestive Solution Product Description

Product Information	Cat.No.	Amount	Storage
hMSC Mild Digestion Solution	SN-02-0040	100 ml	2°C - 8°C

III. Instructions (Using a T75 culture flask digestion as an example)

Table 2: Recommended Reagent Volumes for hMSC Passaging and Culture Operations

Culturing Vessel	Growth Area	ncMission Complete Medium	Trypsin/Trypsin Inhibitor
6-Well Plate	9.6 cm^2 /well	2 mL/well	1 mL/well
T75 Culture Flask	75 cm^2	15 mL	5 mL
T175 Culture Flask	175 cm^2	25 mL	8-10 mL
T225 Culture Flask	225 cm^2	35 mL	10-13 mL

1. Choice of Passage Timing: Different hMSCs have different growth rates. It is recommended to select the appropriate passage timing based on cell confluence. Passage can be carried out when the cell confluence reaches approximately 80 - 85%.

2. Take out the required amount of **ncMission hMSC Complete Medium**, DPBS (**without calcium and magnesium**), and hMSC Gentle Dissociation Buffer 30 minutes in advance. Pre-warm DPBS and hMSC Gentle Dissociation Buffer to 37°C, while the ncMission Complete Medium can be left at room temperature.

3. Aspirate the culture medium, rinse the cells once with pre-warmed DPBS (calcium- and magnesium- free), and then add pre- warmed hMSC Digestion Solution (refer to Table 2 for the dosage). Incubate at 37°C for 6~8 minutes. Remove the cell culture flask, gently rock it horizontally, and use a pipette to disperse the cells. Transfer the cells to a centrifuge tube and centrifuge at 250×g for 5 minutes.

4. Aspirate the supernatant, resuspend the cells in 5 mL of physiological saline/DPBS, filter the cell suspension through a 100μm cell strainer, and take a sample for cell counting. The cell viability should be ≥90%.

Centrifuge again to collect the cells at 250×g for 5 minutes.

5. Resuspend the cells in 5 mL of **ncMission hMSC Complete Medium**. Seed the cells into the cell culture vessel at an appropriate density (**6000-8000 cells/cm², with a recommendation of 6000 cells/cm²**), and add the appropriate amount of pre-warmed fresh **ncMission hMSC Complete Medium (refer to Table 4)**. Gently rock the culture vessel in a cross pattern three times, place it in an incubator at 37°C with 5% CO2 concentration and saturated humidity, rock it in a cross pattern three more times, and culture. Continue culturing for 3 days; when the cell confluence reaches **85-90%**, you can choose to passage or cryopreserve the cells.