NcMission™ hMSC Medium V3.0

Product Manual

Catalog#RP02010 1 Kit (525 mL)

1. Product Introduction

NcMission™ hMSC Medium V3.0 is a serum-free, animal component-free complete medium designed for primary human mesenchymal stem cells (hMSCs). This medium supports the stable proliferation of hMSCs, preserving normal surface marker expression ($CD73^+/CD90^+/CD105^+$, $CD14^-/CD34^-/CD45^-/CD79\alpha^-/HLA\text{-}DR^-$) and retaining intact trilineage differentiation potential, including osteogenic, chondrogenic, and adipogenic differentiation.

2. Product Information

Table 1. NcMission™ hMSC Medium V3.0 Product Description

Product Information	Cat.No.	Amount	Storage
NcMission™ hMSC Medium V3.0 contains:	**RP02010**	**1 Kit**	*
NcMission™ hMSC Medium V3.0 Basal Medium	RP02010-1	500 mL	2–8 °C
NcMission™ hMSC Medium V3.0 Supplement（21×）	RP02010-2	25 mL	-80 °C or -20 °C

*Mix the basal medium and supplements to prepare the complete medium, which is stable at 2 °C to 8 °C for up to 2 weeks.

3. Reagents and Materials

Table 2. Reagents & Materials

Reagents & Materials	Brand (e.g.)	Cat.NO. (e.g.)
NcMission™ hMSC Medium V3.0	Shownin	RP02010
hMSC Cryopreservation Medium	Shownin	SN-06-1310
TrypLE Express Enzyme (1×), no phenol red	Thermo Sci.	12604013
T75/T175/T225 Culture Flasks	Thermo Sci.	156499/159910/159934
15 mL/50 mL Centrifuge Tube	Thermo Sci.	N/A
1.5/2 mL Cryovials	Thermo Sci.	N/A
10 μL/200 μL/1000 μL Pipette Tips	Rainin.	N/A

4. Preparation of Complete Medium

4.1 Thaw NcMission™ hMSC Medium V3.0 Supplement at 4 °C, and **do not thaw at 37 °C.**

4.2 In a biosafety cabinet, use a sterile pipette to mix the following two components to prepare the complete medium.

NcMission™ hMSC Medium V3.0 Basal Medium: 500 mL

NcMission™ hMSC Medium V3.0 Supplement: 25 mL

4.3 The complete medium can be stored at 2–8 °C and should be used within 2 weeks.

Tips: The Supplement can be aliquoted based on actual requirements and stored frozen. For instance, it can be aliquoted into 5 vials, each containing 5 mL. Prior to use, thaw 5 mL of the supplement and combine it with 100 mL of basal medium to formulate the complete medium, which must be used within 2 weeks. The supplement should not be subjected to more than 2 freeze-thaw cycles.

5. Isolation and Preparation of Primary hMSCs (Using Adipose Tissue as an Example)

5.1 Adipose Tissue Collection: Collect adipose tissue according to clinic protocols. Transport it at 4 °C and process within 24 hours.

5.2 Under aseptic conditions, aspirate the lipoaspirate. Wash the tissue several times with DPBS (or physiological saline) to remove drugs used during liposuction and blood cells until the wash solution is no longer blood-colored. Using sterile ophthalmic scissors and forceps, clean the tissue and mince it into pieces of approximately 1–2 mm^3.

5.3 Digest with 0.1% Type II collagenase at 37 °C with agitation for 45–60 minutes.Centrifuge the digest at 800 × g for 10 minutes. The upper layer consists of undigested adipose tissue and oil. Carefully insert a pipette into the lower layer and aspirate the cell-containing digestate. Filter the digestate through a 70 μm cell strainer. Centrifuge the filtrate at 600 × g for 8 minutes. Discard the supernatant. Resuspend the cell pellet in a volume of DPBS (or physiological saline) equivalent to twice the pellet volume, and centrifuge at 600 × g for 5 minutes. Repeat this centrifugation wash step under the same conditions once more.

5.4 Add NcMission™ hMSC complete medium, adjust the density to 2 × 10^4 cells/mL. Seed cells into a T25 cm^2 flask. Maintain culture in a incubator with 37 °C, 5% CO_2 and the humidified environment.

5.5 Replace with fresh medium after 48 hours, and then change the medium every 3 days thereafter. Document each medium replacement. When the cell layer reaches 90% confluence, collect the cells.

5.6 Digestion: Aspirate the supernatant and tissue pieces, add physiological saline for washing once and then aspirate and discard. Add TrypLE pre-warmed to 37 °C(refer to Table 4 for the volume of digestion solution) and digest at 37 °C for 5–7 minutes (do not move midway). Subsequently, add an equal volume of NcMission™ hMSC complete medium (or physiological saline) to terminate digestion. Collect the cells and centrifuge at 200 × g for 5 minutes.

5.7 Counting: Resuspend the cells in 5–10 mL of physiological saline, filter through a 100 μm cell sieve once, and take a sample for counting. The recommended viability may be ≥ 90%. Collect the cells by centrifugation (200 × g, 5 minutes).

5.8 Seeding: Resuspend the cells in 5 mL of NcMission™ hMSC complete medium. Seed the cells into cell culture vessels at an appropriate density (6000–8000 cells/cm^2). Add pre-warmed NcMission™ hMSC complete medium(refer to Table 3 for the volume of complete medium). Gently rock the vessel horizontally 3 times and place the vessel in an incubator at 37 °C with 5% CO_2 and saturated humidity. Culture for 3 consecutive days; when the cells reach 80–85% confluence, passaging may be performed as required.

5.9 Cryopreservation: If cell cryopreservation is required, after centrifugation in step 5.6, add cryopreservation solution to resuspend the cells at a certain density (e.g., 2 × 10^6 cells per vial). Transfer to a programmable cooling box, freeze at -80 °C overnight, and store in liquid nitrogen the next day.

6. Thawing hMSCs (Using a T75 Flask as an Example; the Procedure is Also Applicable to Other Vessels)

6.1 Preheat a water bath to 37 °C. Warm an appropriate amount of NcMission™ hMSC complete medium to room temperature.

6.2 Retrieve cryopreserved cells from dry ice and thaw in a 37 °C water bath until only small ice crystals remain.

6.3 Transfer the cell suspension to a 15 mL centrifuge tube. Add 10 mL of pre-warmed NcMission™ hMSC complete medium dropwise and mix gently. Centrifuge at 200 × g for 5 minutes.Resuspend the cells in 5 mL of NcMission™ hMSC complete medium and count.

6.4 Seed the cells into the vessel at an appropriate seeding density (6000–8000 cells/cm²), and add an appropriate amount (refer to Table 3) of fresh NcMission™ hMSC complete medium that has been equilibrated to room temperature. Gently rock the flask horizontally 3 times and incubate in an incubator at 37 °C with 5% CO_2 and saturated humidity. When the cells reach 80–85% confluence, passaging may be performed as required.

Table 3. Recommended Reagent Usage for hMSC Passaging and Maintance

Culture Vessel	Growth Area	NcMission™ hMSC Complete Medium	TrypLE
6-Well Plate	9.6 cm^2/well	2 mL/well	1 mL/well
T75 Flask	75 cm^2	15 mL	4 mL
T175 Flask	175 cm^2	25 mL	8 mL
T225 Flask	225 cm^2	35 mL	10 mL

7. Passaging and Cryopreservation of hMSCs (Using T75 Flask as an Example; the Procedure is Also Applicable to Other Vessels)

7.1 Selection of Passaging Timing: The growth rates of hMSCs may vary; therefore, it is recommended to determine the appropriate passaging time based on cell confluence. Passaging may be performed when the cells reach approximately 80–85% confluence.

7.2 Remove the NcMission™ hMSC complete medium and digestion solution (Research-grade: Trypsin solution + Trypsin inhibitor; Clinical-grade culture: TrypLE) from storage 30 minutes in advance and allow them to equilibrate to room temperature..

7.3 Detachment: Aspirate the medium, wash with DPBS (without Ca^{2+} /Mg^{2+}), and add pre-warmed (37 °C) TrypLE（referring to Table 3）. Incubate at 37 °C for 5-7 minutes (do not move midway). Neutralize with an equal volume of NcMission™ hMSC complete medium. Centrifuge at 200 × g for 5 minutes.

7.4 Counting: Resuspend the cells in 5 mL of saline, filter through a 100 μm strainer, and count. Cell viability should be ≥ 90%. Centrifuge again at 200 × g for 5 minutes.

7.5 Seeding: Resuspend the cells in 5 mL of NcMission™ hMSC complete medium and seed at 6000–8000 cells/cm². Add pre-warmed fresh NcMission™ hMSC complete medium（referring to Table 3）. Gently rock the flask horizontally 3 times and incubate in a 37 °C, 5% CO_2 incubator. Maintain culture for 3 days until 80–85% confluent before passaging.

7.6 Cryopreservation: If cell cryopreservation is required, after step 7.3, add cryopreservation solution to resuspend the cells at a certain density (e.g., 2 × 10^6 cells per vial). Transfer to a programmable cooling box, freeze at -80 °C overnight, and store in liquid nitrogen the next day.

8. Adaptation of hMSCs from Other Medium Systems to NcMission™ hMSC Medium V3.0

When transitioning to NcMission™ hMSC Medium V3.0, recover or passage the cells in their original medium. On day 1, replace the medium with NcMission™ hMSC Medium V3.0. After one passage, the cells will adapt to the new system.