NcMission™ hMSC Medium V3.0

Product Manual

I. Product Introduction

NcMission™ hMSC Medium is a serum-free, animal component-free complete medium designed for the culture of primary human mesenchymal stem cells (hMSCs). hMSCs cultured in this medium exhibit stable proliferation, maintain normal expression of surface markers (CD73+/CD90+/CD105+, CD14-/CD34-/CD45-/CD79α-/HLA-DR-), and retain their trilineage differentiation potential (osteogenic, chondrogenic, and adipogenic differentiation).

II. 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**	**2℃～8℃***
NcMission™ hMSC Medium V3.0 Basal Medium	RP02010-1	500 mL	2℃～8℃
NcMission™ hMSC Medium V3.0 Supplement（21×）	RP02010-2	25 mL	-20°C or -80°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.**

III. Reagents and Materials

Table 2: Reagents & Materials

Reagents & Materials	Brand (e.g.)	Cat.NO. (e.g.)
NcMission™ hMSC Medium	Shownin	RP02010
MSC Cryopreservation Medium	Shownin	RP02004
0.25% Trypsin Solution	Shownin	RP02011
Trypsin Inhibitor	Shownin	RP02012
TrypLE Express Enzyme (1X), 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
Freezing Container	Thermo Sci.	5100-0001

IV. Preparation of Complete Medium

4.1 Thaw NcMission™ hMSC Medium V3.0 Supplement (21×) 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 culture 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°C–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 MSC serum-free supplement and combine it with 100 mL of MSC basal medium to formulate the complete medium, which must be used within 2 weeks. The MSC serum-free supplement should not be subjected to more than 2 freeze-thaw cycles.

V. Isolation and Culture of Primary hMSCs (Using Umbilical Cord Tissue Explant Method as an Example)

5.1 Umbilical Cord Collection: Collect the umbilical cord and place it in NcMission™ Basal Medium. Transport at 4°C and process within 24 hours.

5.2 Material Preparation: Prepare freshly made NcMission™ hMSC Medium, sterile culture dishes (6-10), medical-grade disinfectant alcohol, physiological saline, a tool kit (2 pairs of scissors, 2 pairs of forceps), and the umbilical cord in preservation solution. Transfer all materials to the biosafety cabinet.

5.3 Umbilical Cord Disinfection: Aspirate the preservation solution and submerge the cord in 75% medical-grade alcohol for 2 minutes.

5.4 Umbilical Cord Washing: Transfer the cord to a sterile dish and wash 2-3 times with physiological saline to remove residual blood.

5.5 Umbilical Cord Sectioning: Cut the cord into 2-3 cm segments and wash again with saline to remove residual blood.

5.6 Umbilical Cord Dissection: Cut along the vein to remove the venous wall, then remove the two arteries. Carefully separate Wharton's jelly, avoiding the epithelium.

5.7 Weighing: Transfer the Wharton's jelly to a 50 mL centrifuge tube, add 3-5 drops of saline to keep it moist, and mince into 2-3 mm^3 pieces. Weigh the tissue.

5.8 Seeding: Resuspend the minced tissue in NcMission™ hMSC Medium and seed into culture flasks according to Table 3. Incubate at 37°C, 5% CO_2, and saturated humidity.

5.9 First Medium Change: On day 5, tilt the flask to allow tissue explants to settle. Aspirate the supernatant and add fresh pre-warmed NcMission™ hMSC Medium. Return to the incubator.

5.10 Second Medium Change: On days 9-10, repeat the medium change as above.

5.11 Passaging: Around day 12, passage the cells. Approximately 2-3×10^6 cells/ T75 flask can be collected from 0.5g of Wharton's jelly.

5.12 Cell Detachment: Aspirate the medium and tissue explants. Wash once with saline and aspirate. Add pre-warmed digestion solution (0.125% trypsin or TrypLE™) and incubate at 37°C for 4-5 minutes. Neutralize with an equal volume of trypsin inhibitor or NcMission™ hMSC Medium. Centrifuge at 200×g for 5 minutes.

5.13 Cell Counting: Resuspend the cells in 5-10 mL of saline, filter through a 100 μm cell strainer, and count. Cell viability should be ≥90%. Centrifuge again at 200×g for 5 minutes.

5.14 Cell Seeding: Resuspend the cells in 5 mL of NcMission™ hMSC Medium and seed at a density of 5000-7000 cells/cm^2 (recommended 6000 cells/cm^2). Add pre-warmed medium according to Table 4. Gently rock the flask horizontally three times and return to the incubator. Culture for 3 days until 80-85% confluent before passaging.

5.15 Cell Cryopreservation: Resuspend cells in cryopreservation medium at 2×10^6 cells/mL. Transfer to a programmable cooling box, freeze at -80°C overnight, and store in liquid nitrogen the next day.

Table 3: Recommended Reagent Usage for Primary MSC Isolation Using Tissue Explant Method

Step	T75 Flask	T175 Flask	T225 Flask
Wharton's Jelly Weight	0.5 g	1 g	1.5 g
Medium for Seeding	10 mL	15 mL	20 mL
1st Medium Change (Day 5)	13 mL	20 mL	30 mL
2nd Medium Change (Days 9-10)	15 mL	25 mL	35 mL

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

6.1 Preheat a water bath to 37°C. Warm an appropriate amount of NcMission™ hMSC 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 Medium dropwise and mix gently. Centrifuge at 200×g for 5 minutes.

6.4 Resuspend the cells in 5 mL of NcMission™ hMSC Medium and count. Seed at 5000-7000 cells/cm² (recommended 6000 cells/cm²) in pre-warmed medium. Gently rock the flask horizontally three times and return to the incubator. Culture for 3 days until 80-85% confluent before passaging.

Table 4: Recommended Reagent Usage for hMSC Passaging and Culture

Culture Vessel	Growth Area	NcMission™ hMSC Medium	Trypsin/Trypsin Inhibitor
6-Well Plate	9.6 cm^2 /hole	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

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

7.1 Passaging Timing: Passage cells when they reach 80-85% confluence.

7.2 Preparation: Warm NcMission™ hMSC Medium and digestion solution (0.125% trypsin or TrypLE™) to room temperature.

7.3 Cell Detachment: Aspirate the medium, wash with DPBS (without Ca^{2+}/Mg^{2+}), and add pre-warmed digestion solution. Incubate at 37°C for 4-5 minutes. Neutralize with an equal volume of trypsin inhibitor or NcMission™ hMSC Medium. Centrifuge at 200×g for 5 minutes.

7.4 Cell 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 Cell Seeding: Resuspend the cells in 5 mL of NcMission™ hMSC Medium and seed at 5000-7000 cells/cm² (recommended 6000 cells/cm²). Add pre-warmed medium according to Table 4. Gently rock the flask horizontally three times and return to the incubator. Culture for 3 days until 80-85% confluent before passaging.

7.6 Cell Cryopreservation: Resuspend cells in cryopreservation medium at 2 × 10^6 cells/mL. Transfer to a programmable cooling box, freeze at -80°C overnight, and store in liquid nitrogen the next day.

VIII. Adaptation of hMSCs from Other Culture Systems to NcMission™ hMSC Medium

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