Research-Grade hMSC Line
Product Manual

I. Product Introduction

Primary human mesenchymal stem cells (hMSCs) are derived from umbilical cord tissue and labeled with fluorescent markers using proprietary gene editing technology. Specific gene fragments are inserted into the target genome, enabling efficient expression of fluorescent proteins or luciferase. All cells exhibit normal chromosomal karyotypes and surface marker expression (CD73+/CD90+/CD105+, CD14-/CD34-/CD45-/CD79α-/HLA-DR-). These cells are suitable for in vivo imaging after transplantation, making them ideal for in vitro experiments, drug screening, safety evaluation, and cell transplantation in disease animal models.

II. Product Information

Table 1: Product Description of Research-Grade hMSC Lines

Product Information	Cat.No.	Amount	Description
Research-grade hMSCs	RC02003	1×10^6/tube	Umbilical cord-derived MSCs with stable proliferation.
iMSC-luc-GFP(P2)	RC02011	1×10^6/tube	Luc and GFP gene fragments inserted at the ROSA26 locus, with nuclear green fluorescence and luciferase expression.
iMSC-Anterase2 (P2)	RC02012	1×10^6/tube	Anterase2 gene fragment inserted at the ROSA26 locus, with efficient Anterase2 expression.
ucMSC-luc-GFP(P3)	RC02013	1×10^6/tube	Umbilical cord MSCs with randomly inserted Luc and GFP gene fragments, showing nuclear green fluorescence and luciferase expression.
ucMSC-Anterase2 (P3)	RC02014	1×10^6/tube	Umbilical cord MSCs with randomly inserted Luc and GFP gene fragments, showing nuclear green fluorescence and luciferase expression.

This product is for research use only and is not intended for medical applications such as diagnosis or treatment.

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
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 Tubes	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. Complete Medium Preparation

4.1 Thaw ncMission Supplement (21×) at 4°C. **Do not thaw it at 37°C.**

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

ncMission Basal Medium： 500 mL

ncMission Supplement (21×): 25 mL

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

TIPS: The Supplement can be aliquoted and stored frozen according to usage. For example, aliquot 5 mL×5 tubes. Thaw 5 mL of Supplement and mix with 100 mL of Basal Medium to prepare the complete medium, which should be used within two weeks. The total number of freeze-thaw cycles should not exceed two.

V. Primary MSC Isolation and Culture (Using the Umbilical Cord Tissue Block Method as an Example)

5.1 Umbilical Cord Collection: After collecting the umbilical cord, place it in the umbilical cord preservation solution (ncMission Basal Medium). Transport at 4°C and process within 24 hours.

5.2 Material Preparation: Prepare freshly prepared ncMission hMSC complete medium, several sterile culture dishes (6-10), medical-grade disinfection alcohol, physiological saline, and a tool kit (2 scissors, 2 forceps). Transfer the umbilical cord (in preservation solution) into the biological safety cabinet.

5.3 Umbilical Cord Disinfection: Aspirate the preservation solution and immerse the cord in 75% alcohol for 2 minutes.

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

5.5 Umbilical Cord Cutting: Cut the umbilical cord into small sections of about 2 - 3 cm, and wash it with physiological saline 2 - 3 times again to clean the residual umbilical cord blood.

5.6 Umbilical Cord Separation: Cut open the umbilical cord along the vein and remove the vein wall. After completely removing the vein wall, the umbilical cord will unfold completely. Then remove the 2 arteries. After completely removing the vein and arteries, carefully separate the Wharton's jelly, and pay attention to avoiding the epidermis.

5.7 Weighting: Transfer the separated Wharton’s jelly into a 50 mL centrifuge tube. Add 3-5 drops of physiological saline to keep it moist. Cut the Wharton’s jelly into small pieces of approximately 2-3 mm^3 using curved-tip

surgical scissors, then weigh the tissue.

5.8 Inoculation: Resuspend the cut Wharton's jelly in ncMission hMSC complete medium. Inoculate the tissue into a culture flask according to Table 3 and place it in an incubator (37°C, 5% CO_2, saturated humidity) for culture.

5.9 First Medium Change: On Day 5 after inoculation, when cells begin to migrate from the tissue block, tilt the culture flask upright at a 30-degree angle, allowing the tissue block to naturally settle to one corner. Aspirate the supernatant, slowly add fresh, thawed ncMission hMSC complete culture medium, gently mix, and return the flask to the incubator for continued culture.

5.10 Second Medium Change: On Days 9-10 after inoculation, when the migrated cells show good condition and begin to form a stacked growth pattern, tilt the culture flask upright at a 30-degree angle, allowing the tissue block to naturally settle to one corner. Aspirate the supernatant, slowly add fresh, thawed ncMission hMSC complete culture medium, gently mix, and return the flask to the incubator for continued culture.

5.11 Passage Timing: It can be passaged around DAY 12, and about 2×106 - 3×106 cells T75 (0.5 g Wharton's jelly) can be collected.

5.12 Cell Dissociation: Aspirate the culture supernatant and tissue block, wash it once with physiological saline, and aspirate it. Add warmed dissociation solution (research - grade 0.125% Trypsin Buffer, clinical - grade TrypLE (0.5×), the amount of dissociation solution is based on Table 4), dissociate at 37°C for 4 - 5 minutes, and then add an equal volume of enzyme inhibitor/ncMission hMSC complete medium to stop the dissociation. Collect cells by centrifugation (200×g, 5 min).

5.13 Counting: Resuspend cells in 5 - 10 mL saline, filter once through a 100 μm cell strainer, and sample for counting. The cell viability should be ≥90%. Centrifuge to collect cells (200×g, 5 min).

5.14 Seeding: Resuspend cells in 5 mL of ncMission hMSC complete culture medium. Seed the cells into the culture vessel at an appropriate density (5000-7000/cm^2, recommended 6000/cm^2), and add an appropriate amount of fresh ncMission hMSC complete culture medium pre-warmed (refer to Table 4). Gently shake horizontally in a cross pattern three times, place in a 37°C, 5% CO2 incubator with saturated humidity, and shake horizontally in a cross pattern three more times before continuing culture. Culture for 3 days until cells reach 80-85% confluence, then passage as needed.

5.15 Cryopreservation: If cryopreservation is required, after centrifugation in step 5.13, resuspend the cells in cryopreservation medium at a density of $2×10^6$ cells/mL. Transfer the cells to a freezing container, store at -80°C overnight, and then transfer to liquid nitrogen the next day for long-term storage.

Table 3: Recommended Reagent Usage for Primary MSC Isolation by Tissue Block Method

Procedure	T75 Flask	T175 Flask	T225 Flask
Wharton's Jelly	0.5 g	1 g	1.5 g
Medium at Inoculation	10 mL	15 mL	20 mL
First Medium Change (DAY 5)	13 mL	20 mL	30 mL
Second Medium Change (DAY 9 - 10)	15 mL	25 mL	35 mL

VI.Resuscitation (Using T75 Flask as an Example, Applicable to Other Culture Vessels)

6.1 Preheat the water bath to 37°C. Prepare the required volume of ncMission hMSC Complete Medium in advance and allow it to reach room temperature.

6.2 Remove the frozen cells and transport them on dry ice to the cell culture area. Take the cells out of the dry ice and place them into the 37°C water bath. Gently shake to thaw the cells. Once the ice crystals in the cell suspension are nearly dissolved (with only ice crystals the size of mung beans remaining), remove the cells from the water bath.

6.3 Immediately transfer the cell suspension into a 15 mL centrifuge tube. Slowly add 10 mL of ncMission hMSC Complete Medium (at room temperature) and mix gently. Centrifuge at 200×g for 5 minutes to collect the cells. Discard the supernatant and resuspend the cells in 5 mL of ncMission hMSC Complete Medium. Count the cells accurately.

6.4 Seed the cells into the culture vessel at an appropriate density (5000-7000 cells/cm², recommended 6000 cells/cm²). Add the required volume of fresh ncMission hMSC Complete Medium (at room temperature, refer to Table 4). Gently mix the culture vessel horizontally three times. Place the vessel in an incubator at 37°C with 5% CO_2 and saturated humidity. Afterward, mix the vessel horizontally three more times and continue culture. After 3 days, when the cell confluence reaches 80-85%, the cells are ready for passage.

Table 4: Recommended Reagent Usage for hMSC Passaging and Culture

Culture Vessel	Growth Area	ncMission hMSC Complete Medium	Trypsin / Trypsin Inhibitor
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

VII. hMSC Passaging & Cryopreservation (Using T75 Flask as an Example, Applicable to Other Culture Vessels)

7.1 Passaging Time: hMSC growth rates vary. Passage when cell confluence reaches approximately 80-85%.

7.2 Preparation: Take out ncMission hMSC Complete Medium and digestion solution 30 minutes in advance to reach room temperature. For research-grade culture, use trypsin solution with trypsin inhibitor; for clinical-grade culture, use TrypLE.

7.3 Aspirate the medium and wash once with DPBS (calcium- and magnesium-free). Add pre-warmed digestion solution (research-grade: 0.125% trypsin solution; clinical-grade: TrypLE (0.5×), refer to Table 4 for volume). Incubate at 37°C for 4-5 minutes. Add an equal volume of trypsin inhibitor or ncMission hMSC Complete Medium to neutralize digestion. Collect the cells and centrifuge at 200×g for 5 minutes.

7.4 Resuspend the cell pellet in 5 mL of saline. Filter through a 100 µm cell strainer. Take a sample for

counting. Cell viability should be ≥90%. Centrifuge again at 200×g for 5 minutes.

7.5 Resuspend the cell pellet in 5 mL of ncMission hMSC Complete Medium. Seed cells into the culture vessel at an appropriate density (5000-7000 cells/cm², recommended 6000 cells/cm²). Add the required volume of pre-warmed fresh ncMission hMSC Complete Medium (refer to Table 4). Mix by gently shaking in a horizontal cross pattern three times. Incubate at 37°C, 5% CO_2, and saturated humidity. Shake in a horizontal cross pattern three more times before continuing culture. Passage when cell confluence reaches 80-85% (typically after 3 days).

7.6 Cryopreservation: For cryopreservation, after Step 7.3, resuspend the cell pellet in freezing medium at the required density (e.g., 2×10^6 cells/mL). Transfer to a controlled-rate freezing container and store overnight at -80°C. Transfer to liquid nitrogen for long-term storage the following day.

VIII. Adapting hMSC from Other Culture Systems to ncMission Medium

When transitioning hMSC from another culture system to ncMission hMSC Complete Medium, it is recommended to perform thawing or passaging in the original medium first. On Day 1, switch to ncMission hMSC Complete Medium. Full adaptation is typically achieved after one passage.