NcMission™ M30 hMSC Medium (Phenol Red-free)

Product Manual

Catalog#SN-02-0030 1 Kit (525 mL)

Product Introduction

NcMission™ M30 hMSC Medium (Phenol Red-Free) is a **serum-free, animal-free,** complete medium developed by Shownin Biotech Co., Ltd. for primary Human Mesenchymal Stem Cells (hMSCs). hMSCs processed in this medium exhibit stable proliferation, maintain normal expression of cell surface markers ($CD73^+/CD90^+/CD105^+$, $CD14^-/CD34^-/CD45^-/CD79\alpha^-/HLA\text{-}DR^-$), and retain their trilineage differentiation potential (osteogenic, chondrogenic, and adipogenic differentiation).

Product Information

Table 1. Product Description

Product	Cat.No.	Amount	Storage
NcMission™ M30 hMSC Medium (Phenol Red-free) contains:	**SN-02-0030**	**1 Kit**	*
NcMission™ M30 hMSC Medium Basal Medium (Phenol Red-free)	SN-00-0003	500 mL	2 °C ~ 8 °C
NcMission™ M30 hMSC Medium Supplement	SN-02-0031	25 mL	-80 °C or -20 °C

*Mix the Basal Medium and the Supplement to prepare the complete medium. It can be stored at 2 °C to 8 °C and should be used within two weeks.

Reagents and Materials

Table 2. Recommended Reagents & Materials

Product	Brand (e.g.)	Cat.No. (e.g.)
NcMission™ M30 hMSC Medium (Phenol Red-free)	Shownin	SN-02-0030
hMSC Cryopreservation Medium	Shownin	SN-06-1310
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 Containerr	Thermo Sci.	5100-0001

Complete Medium Preparation

1. Thaw NcMission™ M30 hMSC Medium Supplement at 4 °C, and **do not thaw at 37 °C.**

2. In a biosafety cabinet, mix the following components using sterile pipettes to prepare the complete medium:

   NcMission™ M30 hMSC Medium Basal Medium (Phenol Red-free) : 500 mL

   NcMission™ M30 hMSC Medium Supplement : 25 mL

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

   Tips: The supplement can be aliquoted and stored frozen according to usage. For example, aliquot 5 mL × 5 vials. Before use, thaw 5 mL of the supplement and mix with 100 mL of basal medium to prepare the complete medium, which should be used within 2 weeks. The supplement should not undergo more than two freeze-thaw cycles.

Isolation and Preparation of Primary MSCs (Using Umbilical Cord Tissue Explant Method as an Example)

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

2. Material Preparation: Prepare freshly made **complete medium**, sterile culture dishes (6–10 units), medical-grade 75% ethanol, 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.

3. Disinfection: Aspirate the preservation solution and submerge the umbilical cord in 75% ethanol for 2 minutes.

4. Washing: Transfer the umbilical cord to a sterile culture dish and wash 2–3 times with physiological saline to remove residual blood.

5. Cutting: Cut the umbilical cord into 2–3 cm segments and wash again with saline 2–3 times.

6. Separation: Cut along the vein to remove the venous wall, then remove the two arteries. Carefully separate Wharton's jelly, avoiding the epithelium.

7. Weighing: Transfer 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.

8. Seeding: Resuspend the minced tissue in complete medium and seed into culture flasks according to Table 3. Incubate at 37 °C, 5% CO_2, and saturated humidity.

9. First Medium Change: On Day 5, cells will begin to migrate out from the tissue explants. Tilt the culture flask upright at a 30-degree angle to allow the tissue explants to settle naturally in one corner of the flask. Aspirate the supernatant and slowly add fresh, pre-warmed **complete medium**. Gently mix and return the flask to the incubator for continued handling.

10. Second Medium Change: On Days 9–10, the migrated cells will exhibit healthy morphology and begin to form multilayered growth. Tilt the culture flask upright at a 30-degree angle to allow the tissue explants to settle naturally in one corner of the flask. Aspirate the supernatant and slowly add fresh, pre-warmed **complete medium**. Gently mix and return the flask to the incubator. Maintain culture.

11. Passaging: Around Day 12, cells can be passaged. Approximately 2–3 × 10^6 cells/T75 flask (from 0.5 g Wharton's jelly) can be collected.

12. Detachment: Aspirate the medium and tissue explants, wash once with saline, and add pre-warmed hMSC Mild Digestion Solution (refer to Table 4). Incubate at 37 °C for 4–5 minutes, then add an equal volume of **complete medium** to neutralize the digestion. Centrifuge at **200 × g for 5 minutes**.

13. 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**.

14. Seeding: Resuspend the cells in 5 mL of the **complete medium** and seed at a density of **5000–7000 cells/cm² (recommended 6000 cells/cm²)**. Add the pre-warmed **complete medium** according to **Table 4**. Gently rock the flask horizontally 3 times, then incubate at 37 °C, 5% CO_2, and saturated humidity. Rock the flask again 3 times and maintain culture for 3 Days. Passaging may be performed when the cells reach approximately 80–85% confluence.

15. Cryopreservation: If cryopreservation is required, resuspend the cells in cryopreservation medium at a density of 2 × 10^6 cells per tube after centrifugation. Transfer to a programmable freezing container, freeze at -80 °C overnight, and then store in liquid nitrogen.

Table 3. Recommended Reagent Usage for Primary MSC Isolation by Explant Method

Step	T75 Culture Flask	T175 Culture Flask	T225 Culture 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 (Day 9–10)	15 mL	25 mL	35 mL

Thawing and recovery of hMSCs

(Using a T75 Flask as an example. The procedure is also applicable to other vessels)

1. Preheat a water bath to 37 °C. Allow the **complete medium** to reach room temperature.

2. Retrieve the cryopreserved cells and transport them on dry ice to the cell culture room. Retrieve the cryopreservation vial from dry ice and place them in a 37 °C water bath. Gently agitate the vial until the ice crystals in the cell suspension are nearly completely dissolved (with only small, pea-sized ice crystals remaining). Remove the vial.

3. Transfer the cell suspension to a 15 mL centrifuge tube and add 10 mL of room temperature **complete medium** dropwise. Gently mix. Centrifuge at 200 × g for 5 minutes, aspirate the supernatant, and resuspend in 5 mL of **complete medium**. Count the cells.

4. Seed the cells at 5000–7000 cells/cm² (recommended 6000 cells/cm²) and add fresh room-temperature complete medium according to **Table 4**. Rock the flask horizontally in a cross pattern 3 times, incubate at 37 °C, 5% CO_2, and saturated humidity. Rock the flask horizontally again 3 times and maintain under standard conditions for 3 Days. Passaging may be performed when the cells reach approximately 80–85% confluence.

Table 4. Recommended Reagent Usage for hMSC Passaging and Culturing

Processing Vessel	Growth Area	Complete Medium	Trypsin/Trypsin Inhibitor
6-Well Plate	9.6 cm^2 /hole	2 mL/well	1 mL/well
T75 Culture Flasks	75 cm^2	15 mL	4 mL
T175 Culture Flasks	175 cm^2	25 mL	8 mL
T225 Culture Flasks	225 cm^2	35 mL	10 mL

Passaging and Cryopreserving hMSCs

(Using a T75 Flask as an example. The procedure is also applicable to other vessels)

1. Passaging time: The growth rate of different hMSC varies, and it is recommended to choose the exact time of passaging based on the cell confluence, which can be done at around **80-85%** cell confluence.

2. Preparation: Allow the **complete medium** and hMSC Mild Digestion Solution to reach room temperature.

3. Aspirate the medium, wash with DPBS (without Ca^{2+}/Mg^{2+}), and add pre-warmed digestion solution (refer to **Table 4**). Incubate at 37°C for 4–5 minutes, then neutralize with an equal volume of **complete medium**. Centrifuge at **200 × g for 5 minutes**.

4. Resuspend the cells in 5 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. Resuspend the cells in 5 mL of **complete medium** and seed at **5000–7000 cells/cm² (recommended 6000 cells/cm²)**. Add fresh pre-warmed complete medium according to **Table 4**. Rock the flask horizontally in a cross pattern 3 times, incubate at 37 °C, 5% CO_2, and saturated humidity. Rock the flask horizontally again 3 times and maintain culture for 3 Days. Passaging may be performed when the cells reach approximately 80–85% confluence.

6. Cryopreservation: Resuspend the cells at a certain density **(e.g. 2 × 10^6 cells/mL)** in cryopreservation medium after **Step 3**. Transfer to a programmable freezing container, freeze at -80 °C overnight, and then store in liquid nitrogen.

Adaptation of hMSCs from Other Culture Systems to NcMission™ M30 hMSC Medium (Phenol Red-free)

When transitioning hMSCs from other culture systems to **NcMission™ M30 hMSC Medium (Phenol Red-free)**, recover or passage the cells in **the original medium**, then switch to NcMission™ M30 hMSC Medium (Phenol Red-free) on Day 1. The cells should adapt to the new medium after one passage.