hMSC Chondrogenesis Differentiation Kit
Product Manual

I. Product Introduction

The Shownin hMSC-Chondrogenesis Differentiation Kit is designed for efficient chondrogenic differentiation of human mesenchymal stem cells (hMSCs).

II. Product Information

Table 1: hMSC Chondrogenesis Differentiation Kit Product Description

Product Information	Cat.No.	Amount	Storage
hMSC Chondrogenesis Differentiation Kit contains:	**RP02014-B**	**1 Kit**	**2℃～8℃**
Chondrogenesis Differentiation Basal Medium	RP02014-B-01	80 mL	2℃～8℃
Chondrogenesis Differentiation Supplement	RP02014-B-02	20 mL	-20°C to -80°C

*After mixing the basal medium and the supplement to form the complete medium, it can be stored at 2°C - 8°C and should be used up within 2 weeks.

III. Reagents and Materials

Table 2: Reagents & Materials

Reagents & Materials	Brand (e.g.)	Cat.No. (e.g.)
NcMission hMSC Medium V3.0	Shownin	RP02010
Alcian Blue 8GX Solution	OriCell	No.ALCB-10001
4% PFA solution	Biosharp	BL539A
1×DPBS w/o Ca2+ /Mg2+	Thermo Sci.	14190250
6-well Plate	Thermo Sci.	140685
1 mL/5 mL/10 mL/25 mL Pipettes	Thermo Sci.	N/A
15 mL/50 mL Centrifuge Tubes	Thermo Sci.	N/A
10 µL/200 µL/1000 µL Pipette Tips	Rainin.	N/A

IV. Reagent Preparation

(i) Preparation of Complete Medium for hMSC Chondrogenesis Differentiation

1. Thaw the Chondrogenesis Differentiation Supplement at 4°C. Do not thaw it at 37°C.
2. In a biosafety cabinet, use a sterile pipette to mix the following components to prepare 100 mL of complete differentiation medium.

   Chondrogenesis Differentiation Basal Medium: 90 mL

   Chondrogenesis Differentiation Supplement: 10 mL
3. The complete medium can be stored at 4°C and should be used within 2 weeks. Protect from light during storage and use.

   TIPS:The Supplement can be aliquoted and stored frozen according to actual usage. The total number of freeze-

thaw cycles should not exceed 2.

V. Chondrogenesis Differentiation of MSCs

(i) MSCs Culture

1. **Culture and preparation of hMSCs,** please refer to the product manual of **NcMission hMSC Medium V3.0** in detail.
2. Culture hMSCs in **NcMission hMSC Medium V3.0**. Seed hMSCs at a density of 5000~10000 cells/cm² in a 6-well plate. Gently rock the plate in a cross pattern three times and place in a 37°C, 5% CO_2, humidified incubator. Rock the plate again three times and continue culturing.

(ii) Chondrogenesis Differentiation of MSCs

1. Granulation formation: When hMSCs reach aproximately 80% confluence:
   1） Digest cells with 1 mL/well of 0.5×TryPLE at 37°C for 3 minutes.
   2） Balance the tubes and centrifuge at 250×g for 5 minutes (acceleration 3, deceleration 7). Aspirate the supernatant and resuspend cells in NcMission hMSC Medium. Count the cells.
   3） Transfer 2×10⁵ cells into a 15 mL centrifuge tube (2 tubes per group). If the cell suspension volume is less than 1 mL, add NcMission hMSC Medium to 1 mL. Balance and centrifuge at room temperature (300×g for 5 minutes; acceleration 3, deceleration 7).
   4） Aspirate the supernatant, gently tap the tube to disperse the cell pellet, and resuspend in 1 mL of complete medium per tube. Balance and centrifuge at room temperature (450×g for 10 minutes).
   5） Loosen the cap and incubate at 37°C, 5% CO_2 for 16 hours (overnight).
2. Induction of Chondrogenesis (Day 1): Replace the NcMission hMSC Medium with the **hMSC Chondrogenic Differentiation Complete Medium**. Carefully aspirate the NcMission hMSC Medium without disturbing the pellets. Slowly add 2 mL of chondrogenic medium per tube along the wall.
3. Continuous culture (10-20 days): Culture for 10-20 days. Change **hMSC Chondrogenic Differentiation Complete Medium** every 2 days, 2mL/tube. Avoid aspirating MSC pellets during the process (14 days of continuous culture is sufficient for rapid cartilage formation).
4. Fixation, dehydration, staining, microphotography:
   1） Fixation and dehydration: Aspirate the medium and wash the pellets with 2 mL/tube of DPBS. Aspirate DPBS and fix with 2 mL/tube of 4% PFA at 25°C for 10 minutes.
   2） Frozen Sectioning: Embed MSC pellets in embedding medium and section at 10 μm thickness (embedded samples can be stored at -80°C for up to 2 months).
   3） Washing: Soak slides in DPBS and wash twice on a shaker (10 minutes each with an appropriate speed).
   4） Staining: After drying, apply OriCell Alcian Blue 8GX Solution to each section, ensuring full coverage. Place slides in a humidity chamber and stain at 37°C for 30 minutes.
   5） Cleaning and Photography: Rinse slides under slow running water for 3 minutes. Dry and observe under a microscope. Avoid direct water flow on the sample to prevent detachment.