hMSC Osteogenic Differentiation Kit
Product Manual

I. Product Introduction

The hMSC Osteogenic Differentiation Kit has an efficient osteogenic directed differentiation capability and can be used for the induced differentiation of human mesenchymal stem cells to osteoblasts.

II. Product Information

Table 1: hMSC Osteogenic Differentiation Kit Product Description

Product Information	Cat.No.	Amount	Storage
hMSC Osteogenic Differentiation Kit contains:	**RP02014-C**	**1 Kit**	**2℃～8℃**
Osteogenic Differentiation Basal Medium	RP02014-C-1	80 mL	2℃～8℃
Osteogenic Differentiation Supplement	RP02014-C-2	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	Shownin	RP02010
2% Alizarin Red S Solution	Sciencell	0223
1×DPBS w/o Ca^{2+} /Mg^{2+}	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 Osteogenic Differentiation

1. Thaw the Osteogenic 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.

   Osteogenic Differentiation Basal Medium: 90 mL

   Osteogenic Differentiation Supplement: 10 mL
3. The complete medium can be stored at 4°C and should be used within 2 weeks.

   TIPS:The Supplement can be aliquoted and stored frozen according to actual usage. The total number of freeze-thaw cycles should not exceed 2.

(ii) Preparation of Alizarin Red Working Solution

1. The Alizarin Red stock solution has a concentration of 2% and is stored at room temperature. Before use, dilute it with deionized water at a ratio of 1:20 (Alizarin Red stock solution: deionized water).
2. Dilute it to a 0.1% working solution. The working solution should be a light brown, clear liquid.

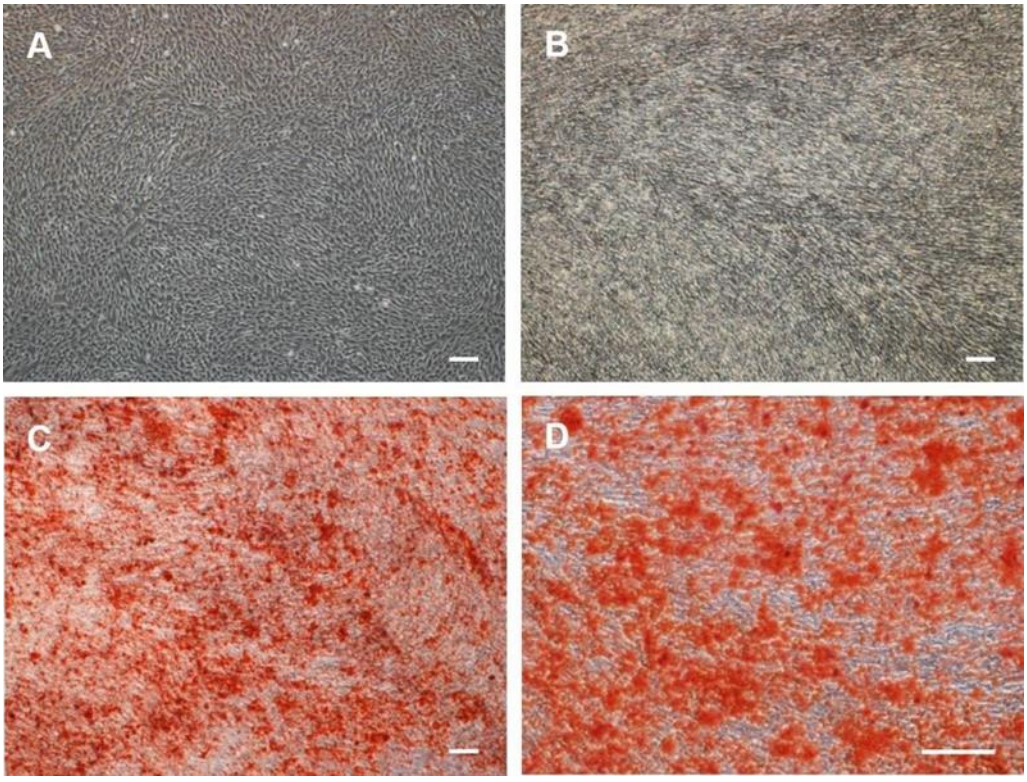
V. Osteogenic Differentiation of MSCs

(i) Culture of MSCs

1. **Culture and preparation of hMSCs,** please refer to the product manual of **NcMission hMSC Medium** in detail.
2. Culture hMSCs in **NcMission hMSC Medium**. 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) Osteogenic Differentiation of MSCs

1. When the confluence of hMSCs reaches approximately 85%, initiate the differentiation process. Aspirate the supernatant and set up an experimental group and a control group. Add the **hMSC Osteogenic Differentiation Complete Medium** to the experimental group, and add **NcMission hMSC Medium** to the control group.
   TIPS: To reduce cell edge detachment or floating during osteogenic differentiation, it is recommended to use Matrigel-coated culture plates. (Matrigel coating protocol: https://www.shownin.com/video.html)
2. Change the medium every 3-4 days, with 2-3 mL per well each time, and continue culturing until the 21st day.
3. On the 21st day, aspirate the supernatant, and add fixing solution (**4% paraformaldehyde**) to fix the cells for 30 minutes.
4. Aspirate the supernatant from both the differentiation group and the control group, add an appropriate volume of **Alizarin Red Working Solution**, a incubate at room temperature and away from light for 20~60 minutes, then aspirate the staining solution, wash with saline or DPBS until no background color is seen, then add saline or DPBS to infiltrate each well, and then observe under the microscope and take pictures.



Morphology of hMSCs during osteogenic differentiation using the hMSC Osteogenic Differentiation Kit.
Scale bar: 200 μm.
A, B: Morphology of cells on Day 1 and Day 21 of differentiation, respectively.
C, D: Morphology of stained cells on Day 21 of differentiation.