hMSC Osteogenic Differentiation Kit

Product Manual

Catalog#RP02014-C 1 Kit

I. Product Introduction

The hMSC Osteogenic Differentiation Kit is intended for use in osteogenic-directed differentiation procedures with human mesenchymal stem cells (hMSCs).

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**	*
Osteogenic Differentiation Basal Medium	RP02014-C-01	80 mL	2–8 °C
Osteogenic Differentiation Supplement	RP02014-C-02	20 mL	-80 °C to -20 °C

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

III. Reagents and Materials

Table 2: Recommended Reagents & Materials

Reagents & Materials	Brand (e.g.)	Cat.No. (e.g.)
NcMission™ hMSC Medium V3.0	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: 80 mL

   Osteogenic Differentiation Supplement: 20 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) Preparation of MSCs

1. 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 3 times and place in a 37 °C, 5% CO_2, humidified incubator. Rock the plate again three times and maintain the culture.

(ii) Osteogenic Differentiation of MSCs

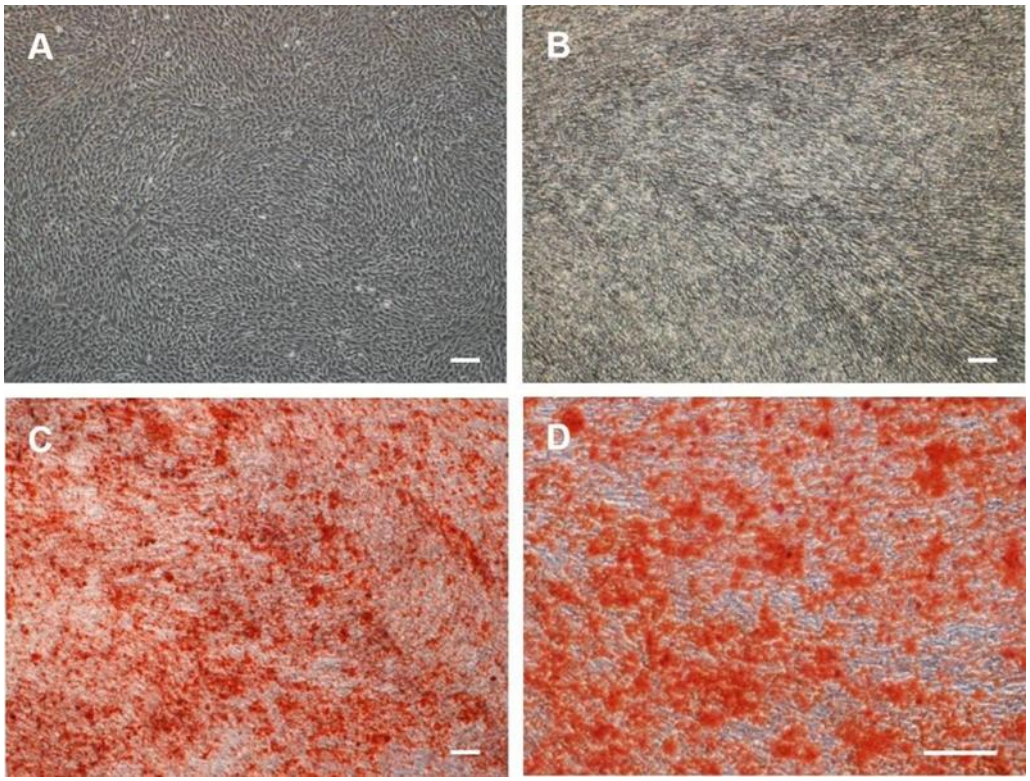
1. When the cell layer reaches approximately 85% confluence, begin the differentiation medium treatment. Aspirate the supernatant and divide the culture into two portions. Add the **hMSC Osteogenic Differentiation Complete Medium** to one portion, and add **NcMission™ hMSC Medium V3.0** to the other portion.

   Tips:To prevent cell edge curling and detachment during the osteogenic differentiation of hMSC, 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, adding 2–3 mL per well each time, and continue the procedure until the 21st day.
3. On the 21st day, aspirate the supernatant, and add fixing solution (**4% paraformaldehyde**) for 30 minutes.

4. Aspirate the supernatant from both portions, add an appropriate volume of **Alizarin Red Working Solution**, and incubate at room temperature protected from light for 20–60 minutes. Remove the staining solution, wash with saline or DPBS for several times, then add saline or DPBS to infiltrate each well. Observe and record the cell status under a microscope.



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.