hMSC Adipogenic Differentiation Kit
Product Manual

I. Product Introduction

The hMSC Adipogenic Differentiation Kit exhibits high - efficiency in adipogenic directed differentiation and can be utilized for the induced differentiation of human mesenchymal stem cells (hMSCs) into adipocytes.

II. Product Information

Table 1: hMSC Adipogenic Differentiation Kit Product Description

Product Information	Cat.No.	Amount	Storage
hMSC Adipogenic Differentiation Kit contains:	**RP02014-A**	**1 Kit**	**2℃～8℃**
Adipogenic Differentiation Basal Medium	RP02014-A-01	80 mL	2°C～8°C
Adipogenic Differentiation Supplement	RP02014-A-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	Shownin	RP02010
Oil Red O	Sigma	O0625
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 Adipogenic Differentiation

1. Thaw the Adipogenic 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.

   Adipogenic Differentiation Basal Medium: 90 mL

   Adipogenic 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 Oil Red O Working Solution

1. **Oil Red O Saturated Solution:** 18 mg of Oil Red powder + 50 ml of isopropanol, stored at room temperature.

2. **Oil Red O Working Solution:** Dilute the stock solution with physiological saline at a ratio of 6:4 (stock solution: physiological saline). Check if there are any precipitated granules in the diluted solution. If so, filter them out using a 0.22 μm filter membrane. Prepare the working solution as needed.

V. Adipogenic 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) Adipogenic 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 Adipogenic Differentiation Complete Medium** to the experimental group, and add **NcMission hMSC Medium** to the control group.
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 **Oil Red O Working Solution**, and incubate at room temperature in the dark for 20 to 60 minutes. Then, aspirate the staining solution, and wash with physiological saline or DPBS until the background color is no longer visible. Add physiological saline or DPBS to each well for immersion, observe under a microscope, and take photos.

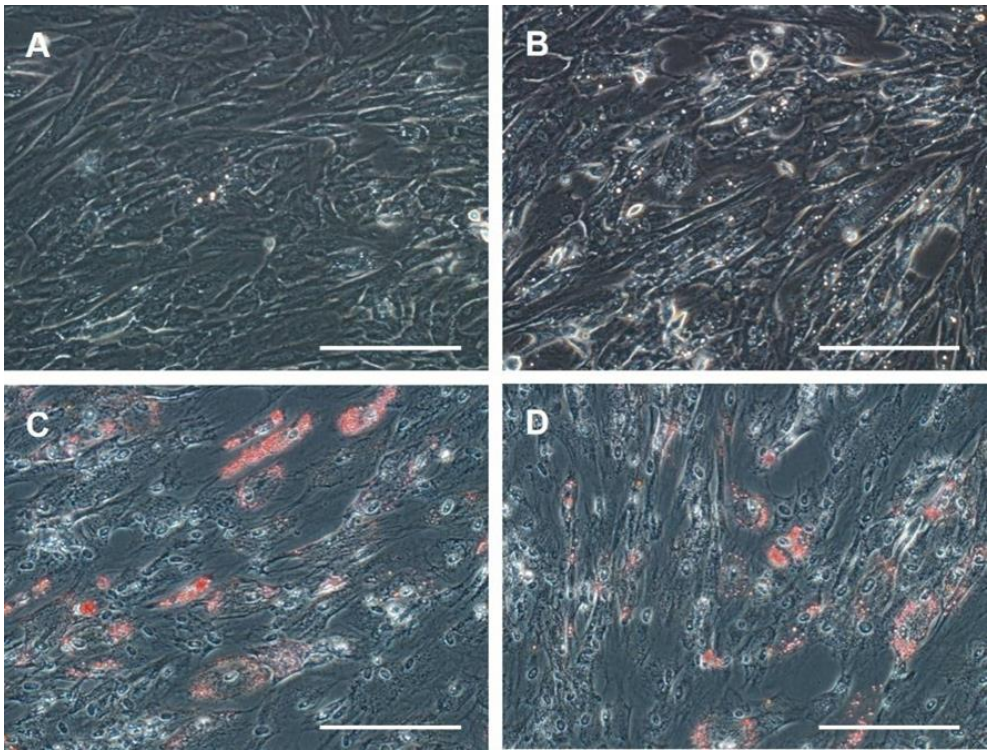

Figure A and B show the cell morphology on Day 10 and Day 21 of differentiation using the hMSC Adipogenic Differentiation Kit.
Figure C and D show Oil Red O staining results on Day 21 of differentiation with the same kit.
Scale bar: 200 μm.