Cat. #. MK430

For Research Use

TaKaRa

Osteoblast-Inducer Reagent (for animal cell)

Product Manual

v201008

Table of Contents

I. Description

Bone tissue consists of osteoclasts, which are responsible for bone resorption, and osteoblasts and osteocytes, which are responsible for bone formation. These cell groups work in concert to maintain bone strength and elasticity.

This product contains a set of osteoblast-inducer reagents, including hydrocortisone, β-glycerophosphate, and ascorbic acid. These reagents induce the efficient differentiation of bone marrow-derived cells and adipose-derived stem cells (mesenchymal stem cells) into osteoblasts when added to culture medium.

II. Kit Components (for 500 ml of medium)

(1) Ascorbic Acid	5 ml
(2) Hydrocortisone	1 ml
(3) β-Glycerophosphate	5 ml×2

III. Materials Required but not Provided

1. Reagents

- RPMI 1640 medium supplemented with glutamine [e.g., RPMI 1640 with L-Glutamine (Lonza Cat. #12-702F)]
- Inactivated fetal bovine serum
- Antibiotics (penicillin and streptomycin) [e.g., Penicillin-Streptomycin Mixture (Lonza Cat. #17-602E)]
- 70% ethanol for disinfection

2. Materials

- Sterilized pipettes
- Dishes and multi-well plates for culturing adherent cells

IV. Storage

- 20°C

V. Protocol

1. Preparation of osteoblast-differentiation medium

- Allow reagents to thaw at room temperature or in a 37°C incubator.
- Sterilize the surfaces of bottles containing media and reagents by spraying or swabbing with 70% ethanol.
- On a clean bench, add (1) Ascorbic Acid, (2) Hydrocortisone, and (3) β-Glycerophosphate into medium to make, respectively, 1% (v/v), 0.2% (v/v), and 2% (v/v).

Example) When making 100 ml of osteoblast-differentiation medium

(1) Ascorbic Acid	1 ml
(2) Hydrocortisone	200 µl
(3) β-Glycerophosphate	2 ml

Note: Osteoblast-differentiation medium may be stored at 4°C for up to 2 months.

2. Differentiation induction

<For mouse, rat or rabbit bone marrow cells>

- Prepare osteoblast-differentiation medium.
- Seed cells with culture medium and incubate for 3 to 7 days.
- Confirm that cells have adhered to and spread on the bottom of the dish, and then remove the medium and replace with osteoblast-differentiation medium.
- Incubate at 37°C for 14 - 21 days in a 5% CO_2 incubator.
- Replace the entire medium every 7 - 14 days.

<For MC3T3-E1 cells [mouse osteoblast-like cell line]>

- Prepare osteoblast-differentiation medium.
- Seed cells and incubate for 1 to 3 days.
- Confirm that cells have adhered to and spread on the bottom of the dish, and then remove the medium and replace with osteoblast-differentiation medium.
- Incubate at 37°C for 10 - 21 days in a 5% CO_2 incubator.
- Replace the entire medium every 3 - 7 days.

Note: it is recommended to prepare cells cultured in a medium without the Osteoblast-Inducer reagents as a control for differentiation-induced changes in cell morphology.

VI. Experimental Examples

1. Cell staining

- MC3T3-E1 cells were cultured and then induced for 3 days in differentiation media supplemented with:
  - A. (1) Ascorbic Acid
  - B. (1) Ascorbic Acid and (2) Hydrocortisone
  - C. (1) Ascorbic Acid and (3) β-Glycerophosphate
  - D. (1) Ascorbic Acid, (2) Hydrocortisone and (3) β-Glycerophosphate.
- Cells were subsequently stained with alkaline phosphatase using the TRACP & ALP double-stain Kit (Cat. #MK300).

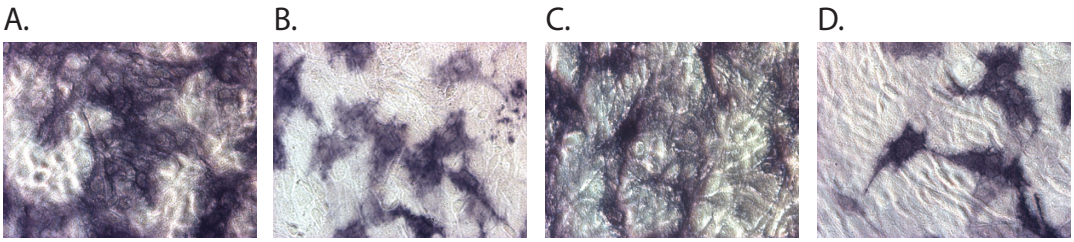

Figure 1. Alkaline phosphatase staining of cells incubated in differentiation medium.

2. Assay of osteocalcin in culture medium.

- Normal rat bone marrow cells [8-week-old, male] were seeded at 1×10^6 cells/well (24-well plates) and incubated for 4 days followed by induction in differentiation medium supplemented with:
  - A. (1) Ascorbic Acid, (2) Hydrocortisone, and (3) β-Glycerophosphate.
  - B. Ascorbic Acid
- Culture medium was sampled over time to assay the level of osteocalcin in the culture supernatant by ELISA using Rat Gla-Osteocalcin High Sensitive EIA Kit (Cat. #MK126).

  Note: Day 0 is the date differentiation medium containing induction reagents was added to cells.
- Alkaline phosphatase staining was performed at the same time using the TRACP & ALP double-stain Kit (Cat. #MK300).

Osteocalcin assay [A_{450}]

	Blank (Medium)	day 1	2	3	7	10	15	21	28
A	0.055	0.058	0.054	0.057	0.071	0.638	3.412	4.062	4.012
B	0.057	0.055	0.058	0.061	0.083	0.070	0.057	0.065	0.062

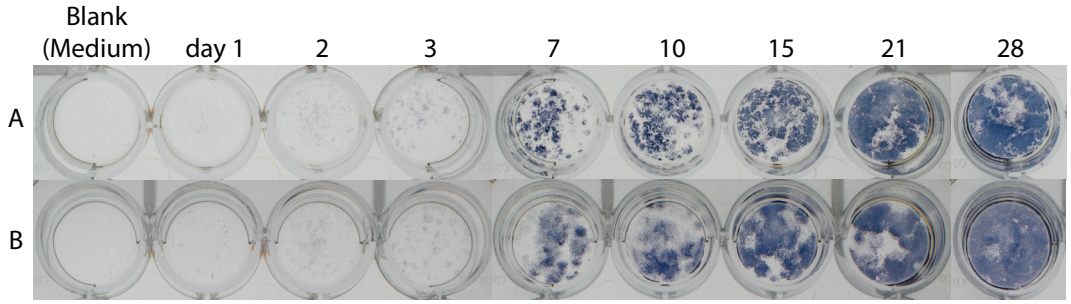

Figure 2. Alkaline phosphatase staining of cells incubated in differentiation medium.

Result: Osteocalcin, a protein secreted by osteoblasts, was detected in the culture medium of differentiated cells.

VII. Related Products

TRACP & ALP double-stain Kit (Cat. #MK300)
TRACP & ALP Assay Kit (Cat. #MK301)
Gla-Type Osteocalcin (Gla-OC) EIA Kit (Precoated) (Cat. #MK111)
Rat Gla-Osteocalcin High Sensitive EIA Kit (Precoated) (Cat. #MK126)