



Calcified Nodule Staining kit

Introduction

Alkaline phosphatase, osteopontin, and osteocalcin are expressed during the differentiation of bone marrow cells into mature osteoblasts and osteoblasts form calcium tubercle. Alizarin Red S, an anthraquinone derivative, has been used to identify Calcified nodule, because Calcium forms an Alizarin Red S-calcium complex, and the products turn (became) red. Calcified Nodule Staining kit (PMC-AK21-COS) contains Substrate-containing Buffer composed mainly of Alizarin Red S and Chromogenic Substrate, and provides a convenient system for staining calcified nodule with ease.

Components/Storage

Component	Quantity	Storage
Substrate-containing Buffer (×100)	60 mL	4°C
Chromogenic Substrate	10 vials	4°C

One kit contains reagents for 10 × 24-well plates

Materials required but not provided

- Phosphate Buffered Saline (PBS)
- methanol
- distilled water

Prepare reagents

- Substrate-containing Buffer

Dilute Substrate-containing Buffer (×100) with distilled water to a 1x concentrated solution.

Note: This working solution can be stored at room temperature for one month.

Protocol < 24well-plate format >

1. Remove culture medium. Wash each well with 1 mL of PBS. (× 3 times)
2. Remove PBS, and fix cells with 500 uL of methanol per well at room temperature for 30 minutes.
 - Methanol should be cooled at -20 °C before use.
3. Remove methanol. Wash each well with 1 mL of distilled water. (× 3 times)
4. Add one vial of Chromogenic Substrate to 10 mL of Substrate-containing Buffer and mix, and then dispense 400 uL of the working solution to each well.
 - This working solution should not be stored. Ready to use.
5. Incubate the plate at room temperature for 5 minutes
6. Wash each well with 1 mL of Substrate-containing Buffer until washing buffer become transparent.

Application example

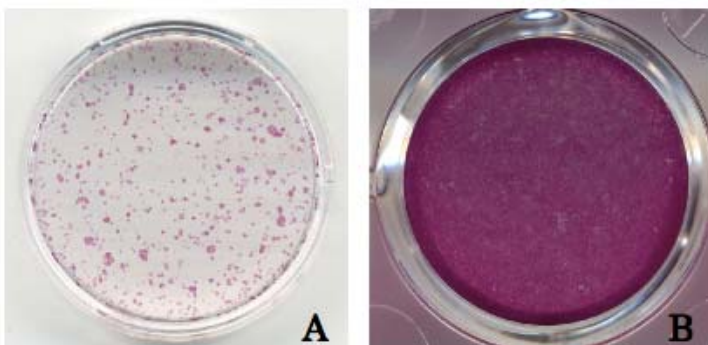


Fig. 1 Cells detected by Calcified Nodule Staining kit

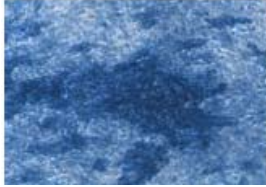
A: 3T3-E1 cells are cultured using Osteogenesis Culture for Osteogenesis Culture kit (Mouse) (Code No. PMC- OGCMO-COS) (35 mm dish)
B: Cells in Osteogenesis Culture Kit (Mouse) (PMC-OGC11-COS) (24well-plate)



Introduction of related kit

1. Alkaline Phosphatase Staining Kit (Cat.No.PMC-AK20-COS)

The Alkaline Phosphatase Staining Kit (Cat.No. PMC-AK20-COS) is used for determining Alkaline Phosphatase activity with ease. The activity of alkaline phosphatase is used as markers for osteoblasts.



Rat osteoblasts (Cat.No. PMC-OBC02-COS) are cultured in Osteogenesis Culture for Osteogenesis Culture kit and the activity of alkaline phosphatase is detected by Alkaline Phosphatase Staining kit (Code: PMC-AK20-COS).

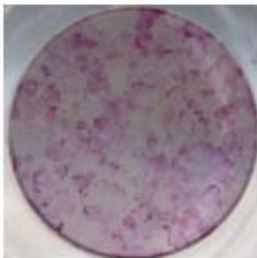
Components

Component	Quantity	Storage
Substrate-containing Buffer	50 mL	4°C
Chromogenic Substrate	10 vials	4°C

One kit contains reagents for 10 × 12-well plates

2. TRAP Staining Kit (Cat.No.PMC-AK04F-COS)

The TRAP Staining Kit (Cat.No.AK04F) is used for the staining of Tartrate-Resistant Acid Phosphatase in osteoclasts. Tartrate-resistant acid phosphate is used as markers for osteoclasts.



Rat osteoblasts (Cat.No. PMC-OBC02-COS) cultured in 96well-plate are detected by TRAP Staining Kit (Cat.No.PMC-AK04F-COS).

Components

Component	Quantity	Storage
Tartrate-containing Buffer	50 mL	4°C
Chromogenic Substrate	10 vials	4°C

One kit contains reagents for 10 × 12-well plates

For research use only. Not for clinical diagnosis.

Manufactured by **Primary Cell Co., Ltd.**



COSMO BIO Co., LTD.

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