



For research use only. Not for clinical diagnosis.

Calcification Staining Kit (Alizarin Red S)

Catalog No. AK21R

June 11, 2026

Introduction

Osteoblasts, the cells responsible for bone formation, are known to increase the expression of markers such as alkaline phosphatase, osteopontin, and osteocalcin over time as differentiation progresses, eventually forming calcified nodules. Alizarin Red S, an anthraquinone derivative, binds to calcium and has long been used as a standard staining method for the direct detection of calcium deposits.

This kit consists of an Alizarin Red S-based staining substrate and buffer, alongside a newly included extraction solution. This configuration not only allows for the simple staining and visual confirmation of calcified nodules but also enables quantitative evaluation through absorbance measurement by using the extraction solution to elute the calcium-bound Alizarin Red S.

Components/Storage

Component	Quantity	Storage
Buffer (×100)	60 mL	Room temperature (Protect from light)
Chromogenic Substrate	10 vials (for 10 mL each)	
Extraction solution	80 mL	

One kit contains reagents for 10 × 24-well plates

Materials required but not provided

- Phosphate Buffered Saline (PBS)
- Methanol
- Distilled water

Preparation of reagents

- Buffer

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

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

Staining Protocol (For 24-well plates)

1. Prepare cells cultured in a 24-well plate.
2. Remove the culture medium. Wash each well with 1 mL of PBS(-) for a total of 3 times.
3. Add 500 µL of methanol per well, and fix the cells at 4 °C for 20 minutes.
4. Note: Methanol should be pre-cooled to -20 °C before use.
5. Remove the methanol. Wash each well with 1 mL of distilled water for a total of 3 times.
6. Add 1 vial of Chromogenic Substrate to 10 mL of Buffer and mix to dissolve completely. Dispense 400 µL of this working solution to each well.
7. Note: The prepared working solution cannot be stored. Prepare immediately before use (ready-to-use).
8. Incubate the plate at room temperature for 5 minutes.
9. Wash each well with 1 mL of Buffer. Repeat washing until the discarded wash buffer becomes clear (transparent).
10. Calcium-deposited areas will be stained red.

Extraction Protocol (For 24-well plates)

1. After staining and washing, completely remove the wash buffer from each well, and add 300 μ L of Extraction Solution.
2. Ensure the Extraction Solution covers the entire surface of the well.
3. Incubate at room temperature for 15 minutes.
4. Collect the supernatant from each well into a microtube.
5. Dispense 100 μ L of the collected sample into each well of a 96-well plate.
6. Measure the absorbance at 560 nm using a microplate reader.

Application example

1. Staining Example

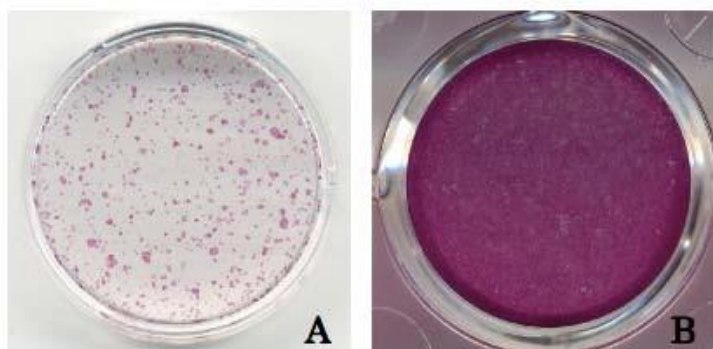


Fig. 1 Cells detected by Calcification

Staining Kit

A: 3T3-E1 cells are cultured using Osteogenesis Culture Medium (Cat No. OGCMO) (35 mm dish)

B: Cells in Osteogenesis Culture Kit (Mouse) (OGC11) (24-well plate)

2. Extraction Example

Inhibition of calcification in cardiac fibroblasts by the addition of pyrophosphate

Pyrophosphate concentration: ① 0 μ M (Control) / ② 0.2 μ M / ③ 1 μ M

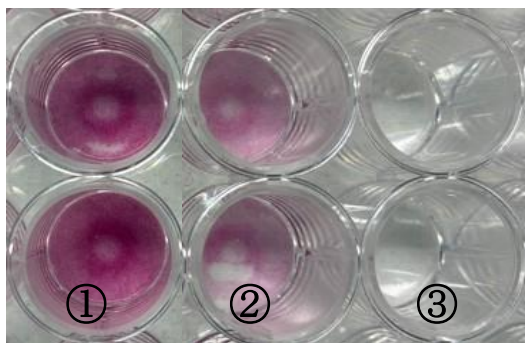


Figure 2. Stained images of calcified cardiac fibroblasts



Figure 3. Extraction solution collected in tubes

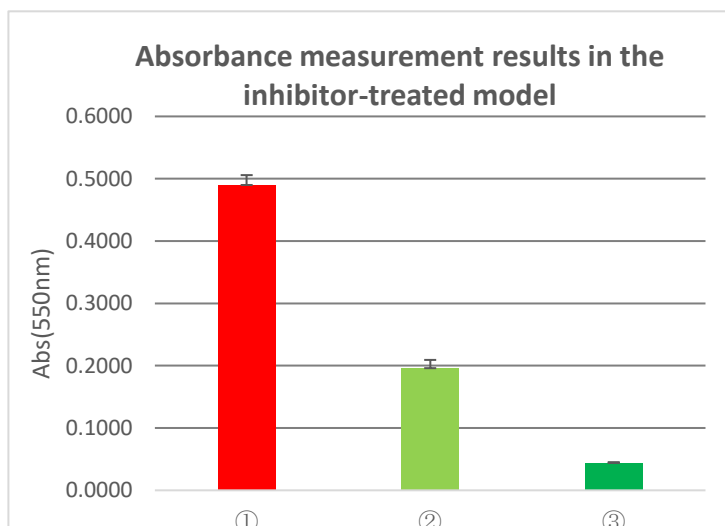
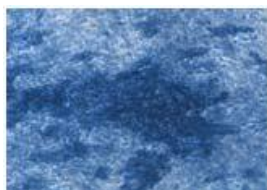


Figure 4. Quantitative evaluation of calcified nodules using the extraction solution

Introduction of related kit

1. Alkaline Phosphatase Staining Kit (Cat.No.AK20)

The Alkaline Phosphatase Staining Kit (Cat. No. AK20) is used for determining Alkaline Phosphatase activity with ease. The activity of alkaline phosphatase is used as a marker for osteoblasts.



Rat osteoblasts (Cat. No. OBC02C) are cultured in Osteogenesis Culture Medium and the activity of alkaline phosphatase is detected by Alkaline Phosphatase Staining kit (Cat No.: AK20).

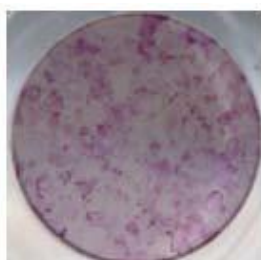
Components

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.AK04F)

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



Rat Osteoclasts (Cat. No. OSC12C) cultured in a 96-well plate are detected by TRAP Staining Kit (Cat. No. AK04F).

Components

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

One kit contains reagents for 10 × 96-well plates

References

- (1) Puchtler et.al., On the history and mechanism of alizarin and alizarin red s stains for calcium. J. Histochem. Cytochem. 17(2) , p110-124(1969)



COSMO BIO Co., LTD.

【JAPAN】

TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,
KOTO-KU. TOKYO 135-0016, JAPAN
Phone: +81-3-5632-9610
FAX: +81-3-5632-9619
URL: <https://www.cosmobio.co.jp/>



COSMO BIO USA

【Outside Japan】

2792 Loker Ave West, Suite 101
Carlsbad, CA 92010, USA
e-mail: support@cosmobiousa.com
Phone/FAX: (+1) 760-431-4600
URL: www.cosmobiousa.com