



Alkaline Phosphatase Staining Kit

Introduction

The Alkaline Phosphatase Staining Kit (Cat.No. PMC-AK20-COS) is used for the staining of alkaline phosphatase in osteoblasts. Bone mass is controlled by the balance between the activity of osteoblasts bone formation and the activity of osteoclasts.

Alkaline phosphatase and tartrate-resistant acid phosphate (TRAP : TRAP staining kit , # PMC-AK04F-COS) are used as markers for osteoblasts and osteoclasts, respectively.

Components

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

One kit contains reagents for 10 × 12-well plates

Additional Materials Required

- 10% Formalin Neutral Buffer Solution
- Phosphate Buffered Saline (PBS)
- Distilled or deionized water

Protocol (12-well plate format)

1. Remove culture medium. Wash each well three times with 1mL of PBS.
2. Add 500uL of the 10% Formalin Neutral Buffer Solution to each well and fix for 20 minutes at room temperature.
3. Remove 10% Formalin Neutral Buffer Solution. Wash each well with 2mL of deionized water. (× 3 times)
4. Dissolve 1 vial of Chromogenic Substrate with 5mL of Substrate-containing Buffer.
5. Add 400uL of Chromogenic Substrates to each well.
6. Incubate at 37□ for 5-20 minutes. Adjust incubation time until stained ALP is clearly showing the result in figure 1.
7. Wash with deionized water to stop the reaction.

Note: Excess incubation will be cause of over stained.

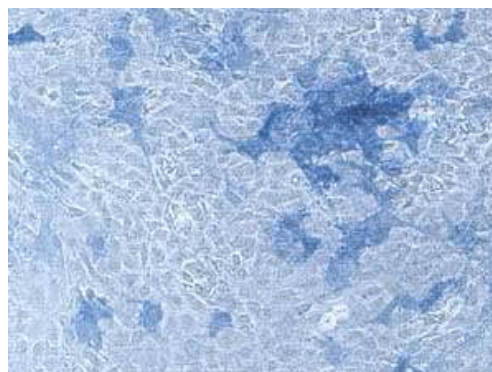


Figure 1: ALP staining of 3T3-E1



References

- (1) A. S. H. De Jong, M. Van Kessel-Van Vark and A. K. Raap.
Sensitivity of various visualization methods for peroxidase and alkaline phosphatase activity in immunoenzyme histochemistry. The Histochemical Journal Volume 17, 1119-1130 (1985)

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