BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Alkaline Phosphatase (ALP) Staining Kit (Cat #: E-103)

COMPONENTS: 20x ALP Buffer: 10 ml, store at room temperature
50x BCIP: 4 ml, store at 4°C
50x Tetrazolium: 4 ml, store at 4°C

PRODUCT DESCRIPTION: Alkaline phosphatase (ALP) is a sensitive biomarker of embryonic stem cells, induced pluripotent stem cells, and osteocytes/odontoblasts. ALP is also a popular reporter enzyme for immunostaining. 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) is a synthetic chromogenic substrate, which upon cleavage by ALP is converted to 5-bromo-4-chloro-3-indole and inorganic phosphate. In the presence of a tetrazolium salt such as NBT or MTT, 5-bromo-4-chloro-3-indole is further converted to an insoluble dark blue diformazan precipitate. Each kit is sufficient for preparation of 200 ml of ALP staining solution. The kit is stable for at least one year if stored and handled properly.

PROTOCOL

Preparation of working solution:

Briefly agitate 20x ALP Buffer before pipetting (cloudy/flocculent appearance is normal). To prepare 1 ml of working solution, add contents in the following order: 0.91 ml dH_2O, 50 µl 20x ALP Buffer, 20 µl 50x BCIP and 20 µl 50x Tetrazolium. Immediately mix contents. The prepared working staining solution can be stored at 4°C for ~2 weeks. Preparation of the solution can be scaled up proportionally for large volume applications.

Staining endogenous ALP

Cells and tissues are briefly (5 min at room temperature) fixed with 4% PFA prior to staining with working solution. Fixed samples are washed with Tris-buffered saline (TBS) prior to incubation with working solution. Remove excess wash buffer. Add sufficient working solution to cover samples. Incubate at 37°C in a humidified chamber until desired dark blue stain appears. Rinse sample with TBS and perform imaging.

Immunostaining:

Tissues section or fixed monolayer cells are incubated with ALP-conjugated secondary antibody and washed with TBS prior to incubation with working solution. Remove excess wash buffer. Add sufficient working solution to cover samples. Incubate at 37°C in a humidified chamber until desired dark blue stain appears. Rinse sample with TBS and perform imaging.

Western blotting:

Western blot should be incubated with ALP-conjugated secondary antibody and thoroughly washed with TBS prior to staining. Add enough working staining solution to cover the blot and incubate at 37°C in a humidified chamber with gentle agitation until desired dark blue band appears. Rinse blot with water and perform imaging.

Additional information:

- BCIP and Tetrazolium solutions are prepared in dimethylformamide and 70% ethanol, respectively. Avoid direct contact with skin and inhalation. Please contact us or visit the product webpage for SDS information on BCIP, Tetrazolium, and dimethylformamide.