# BIOMEDICAL RESEARCH SERVICE CENTER
# UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

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## BCIP Substrate Kit, ALP-based Immunostaining (Cat #: A-123)

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| **COMPONENTS**: | 20x ALP Buffer: | 10 ml, store at room temperature |
| | 50x BCIP Solution: | 4 ml, store at 4℃ |
| | 50x Tetrazolium Solution: | 4 ml, store at 4℃ |

**PRODUCT DESCRIPTION:** Alkaline phosphatase (ALP) and horseradish peroxidase (HRP) are two reporter enzymes widely used in immunoassays due to their stability. In addition, ALP is a sensitive marker of embryonic stem cells and induced pluripotent stem cells. 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) is a synthetic chromogenic substrate, which upon cleavage by alkaline phosphatase 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, thus enabling its use for sensitive ALP-based application and detection of pluripotentstem cells. The working solution can be used for ALP-based in situ immunostaining and Western blotting. The kit is sufficient for preparing 200 ml of ALP staining solution and is stable for at least one year if handled properly.

**PROTOCOL:**

**Preparation of working solution:**

Briefly agitate 20x ALP Buffer before pipetting (cloudy/flocculent appearance is normal). To prepare 1 ml 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 vortex tube thoroughly to mix contents. The prepared working staining solution can be stored at 4°C for several weeks. Preparation of the solution can be scaled up proportionally for large volume application.

**Immunohistochemical staining:**

Deparaffinized issues sections or fixed adherent cells should be incubated with ALP-conjugated secondary antibody and thoroughly washed with Tris-buffered saline (TBS) prior to staining. Remove excess wash buffer. Add enough working staining solution to cover the tissue or cells, and incubate at room temperature (or 37°C) in a humidity chamber until desired dark blue stain develops. Rinse sample with water. The sample is now ready for imaging.

**Western blotting:**

Western blot should be incubated with ALP-conjugated secondary antibody and thoroughly washed with Tris-buffered saline (TBS) prior to staining. Add enough working staining solution to cover the blot and incubate at room temperature (or 37°C) in a humidity chamber with gentle agitation until desired dark blue band appears. Rinse blot with water. The blot is now ready for 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 MSDS information on BCIP, Tetrazolium, and dimethylformamide.
- Addition of 1mM levamisole can be applied to tissue sections to eliminate endogenous ALP activity if necessary.
- TBS is 50 mM Tris-Cl, pH 7.5 and 150 mM NaCl. To prepare, dissolve 6.05 g Tris and 8.76 g NaCl in 800 ml $H_2O$. Adjust pH to 7.5 with 1 M HCl and make volume up to 1 liter with $H_2O$. TBS is stable at 4℃ for several weeks.