# BCIP/NBT Alkaline Phosphatase Color Development Kit

## Product description

Alkaline phosphatase (AP/ALP/AKP/ALKP/ALPase/Alk Phos) is a group of isoenzymes that can generate phosphate ions and free hydroxyl groups by hydrolyzing phosphate monoesters to remove phosphate groups on substrate molecules, and its dephosphorylation substrates include nucleotides, proteins and alkaloids, and are most effective under alkaline conditions. Alkaline phosphates have hydrolase activities, such as the common calf intestinal alkaline phosphatase (CIAP/CIP), which is widely used in the detection of labeled proteins and nucleic acids such as secondary antibodies, as well as for dephosphorylation (dephosphorylation) of DNA or RNA 5' and 3' ends, especially the 5' end of plasmids to avoid plasmid self-linking.

Nitroblue (5-Bromo-4-chloro-3-indolyl phosphate, BCIP) is one of the best substrate combinations for alkaline phosphatase, and Nitroblue tetrazolium chloride (NBT) is a dark blue amorphous microsoluble substance. Catalyzed by alkaline phosphatase, BCIP is hydrolyzed to produce a strongly reactive product that reacts with NBT to form the insoluble dark blue to blue-violet compound NBT-formazan. Strong colors can be observed visually, are very stable, and do not fade when exposed to light.

This kit can be used for immunohistochemical chromography, Western and other membrane chromogenic and identification of induced pluripotent stem cell iPS.

## Components and storage conditions

| Components | K4151-100 mL |
|---|---|
| AP Reaction buffer | 100 mL |
| BCIP | 1 mL |
| NBT | 1 mL |
| Store the components at -20°C away from light for 12 months. | |

## Experimental manipulation

### 1. Sample handling - processing of tissue sections/cell samples/membranes

For tissue sections or cell samples or membranes, after incubation with alkaline phosphatase-labeled antibodies or other forms of probes, select appropriate wash solution to wash 3-5 times for 3-5 min each. For tissue or cell samples that detect endogenous alkaline phosphatase, after sample fixation, also wash 3-5 times

with appropriate wash solution for 3-5 min each.

### 2. Preparation of BCIP/NBT staining solution

Dissolve 1 mL each of NBT and BCIP in 100 mL of the preparation staining solution. *Note: It is recommended to configure according to the amount required.*

### 3. Chromogenic reactions

Step 1After the last wash, remove the wash solution and add an appropriate amount of BCIP/NBT staining solution to ensure adequate coverage of the sample. Incubate at room temperature in the dark for 1~5 min until the color is developed to the expected depth.

### 4. Termination of chromogenic reactions

The color development reaction can be terminated by removing the BCIP/NBT staining solution and then washing 1-2 times with distilled water, or by rinsing with 1% glacial acetic acid.

### 5. Post-processing

The membrane (such as PVDF film, etc.) is air-dried and stored in a dark place in a plastic sleeve. *Note: Dry film can be stored at 2-8 °C in a plastic sleeve between two sheets of transfer paper.*

For tissue sections or cell samples, after the chromogenic reaction is terminated, it can be stained with Neutral Red Staining Solution (K1185 Neutral Red Staining Solution) if necessary for easy observation. For membranes, after the chromogenic reaction is terminated, it can be dried at room temperature and stored in the dark.

## Notes

1. BCIP is irritating to the human body, NBT is harmful to the human body, please pay attention to appropriate protection.
2. Please centrifuge before using BCIP and NBT to avoid losses caused by liquid hanging on the wall.
3. The working liquid should be prepared for immediate use, and the prepared working solution is effective within 1 hour.
4. The amount of working fluid must be sufficient to ensure complete coverage of tissue sheets or blotting membranes.
5. This product is for scientific use only.

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