

## PRODUCT INFORMATION

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### BCIP/NBT ready-to-use substrate for Immunohistochemistry and Blotting

Cat. No.: 15246

#### PRODUCT DESCRIPTION

BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazo-lium) is a substrate used for detection of alkaline phosphatase (AP) in immunohistology and membrane-bound assays e.g. western blots. The reaction product forms a bluish grey to black precipitate. The intensity of the colour is directly proportional to the enzyme concentration.

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#### Direction to use:

The substrate is supplied ready-to-use in 1x concentration. Contains non-toxic-stabilizers. **DO NOT DILUTE.**

Warm to reaction temperature prior to use. Discard if solution is turbid or purple.

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#### Procedure

- After reaction with AP labeled probe, wash tissue sections or blot membrane thoroughly with buffer, e.g. Tris/HCl or TBS containing 0.1 % Tween 20

*DO NOT USE PHOSPHATE BUFFERS. Inorganic phosphate is a powerful inhibitor of AP.*

- Complete cover tissue sections or membrane with BCIP/NBT solution and incubate 5-15 minutes protected from light (longer incubations may be necessary depending upon enzyme activity).
  - Stop the enzyme reaction by thoroughly washing tissue sections or membranes with deionized water
  - If desired, counterstain tissue sections in a 0.5% solution of neutral red that has been prepared in a 0.1 M acetate buffer, pH 5.2. Hematoxylin solutions will not provide sufficient contrast.
  - A very fine blue-purple precipitate will be localized at sites of AP activity on tissue section. Purple bands or dots will be visible at the sites of AP activity on membranes.
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**Storage:** Store the substrate at +2 °C to +8 °C.

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