



BCIP/NBT
Alkaline Phosphatase
Substrate
Cat. No. SK-5400

Store at 2-8 °C
Burlingame, CA USA

BCIP/NBT Substrate

BCIP/NBT Substrate produces an indigo reaction product in the presence of alkaline phosphatase (AP) enzyme. **BCIP/NBT** Substrate can be used on tissues or cells, or on membranes such as nitrocellulose, PVDF, or nylon.

The **BCIP/NBT** Substrate kit contains all of the reagents (except buffer) necessary to prepare the substrate working solution. These reagents are supplied in convenient dropper bottles.

REAGENTS:

- 4 ml Reagent 1
- 3 ml Reagent 2
- 4 ml Reagent 3

STORAGE:

- Store reagents in original bottles at 2-8 °C.
- Avoid storing reagents or working solution in strong direct light.

PREPARATION OF SUBSTRATE WORKING SOLUTION:

- To 5 ml of 100 mM Tris-HCl, pH 9.5 buffer * \diamond
 - Add 2 drops (84 μ l \dagger) of Reagent 1
 - Add 2 drops (40 μ l \dagger) of Reagent 2
 - Add 2 drops (80 μ l \dagger) of Reagent 3
- Mix well before use

* 0.1% Tween® 20 in the BCIP/NBT working solution is recommended for use with antigen retrieved tissues.

\diamond For blots, the buffer volume can be increased to 15 ml.

\dagger Drop volumes differ due to solvent compositions.

INSTRUCTIONS FOR USE:

For Tissues or Cells

After incubation with an AP detection system, rinse well. Incubate with the substrate working solution for 20-30 minutes. Optimal development times should be determined by the investigator. The incubation time of this substrate can be lengthened (up to 24 hours) providing significantly more sensitivity. Improved staining may be obtained by developing the substrate in the dark.

Wash slides in buffer for 5 minutes. Rinse in water and counterstain, if desired (see chart on reverse).

For permanent mounting: Dehydrate, clear and mount in a permanent mounting medium, such as **VectaMount™** (Catalog No. H-5000). **BCIP/NBT** is not compatible with DPX mountant.

For aqueous mounting: Use an aqueous-based mounting medium such as **VectaMount™ AQ** (Cat. No. H-5501).

For Blots

Incubate blot for 20-30 minutes. Longer incubation times will increase sensitivity. When development is satisfactory, rinse membrane with water and air dry.

NOTES:

1. Do not heat **BCIP/NBT** substrate kit components or working solution.
2. Occasionally a precipitate may form in some reagents upon prolonged storage. This will have no effect on the quality or intensity of the staining. Do not filter the reagents or working solution.
3. Endogenous alkaline phosphatase activity (other than the intestinal isoenzyme) can be inhibited by the addition of **Levamisole** (Cat. No. SP-5000) to the buffer prior to the preparation of the working solution. Alternatively, endogenous alkaline phosphatase, including the intestinal isoenzymes, can be inactivated before immunostaining with the use of **BLOXALL™** Blocking Solution (Cat. No. SP-6000) or with other tissue pretreatments (Bulman AS and Heyderman E; J. Clin. Pathol. 34, 1349-1351, 1981).

IMPORTANT: Little is known about the toxicity and carcinogenicity of the substrate kit components. Appropriate care should be exercised when using this reagent including gloves, eye protection, lab coats, and good laboratory procedures. Dispose in accordance with local regulations.

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For Laboratory Use

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