

BCIP/NBT

Alkaline Phosphatase Substrate

Cat. No.	SK-5400
Storage	Store reagents in original bottles at 2-8 °C. Avoid storing reagents or working solution in strong direct light.
Description	This kit contains all of the reagents necessary to prepare the substrate working solution. BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium) Substrate produces an indigo reaction product in the presence of alkaline phosphatase (AP) enzyme.

Components

Product Name	Volume
BCIP/NBT Reagent 1	4 ml
BCIP/NBT Reagent 2	3 ml
BCIP/NBT Reagent 3	4 ml

Preparation of Substrate Working Solution

To 5 ml of 100 mM Tris-HCl, pH 9.5 buffer*:

- Add 2 drops (≈ 84 μl†) of BCIP/NBT Reagent 1
- Add 2 drops (≈ 40 μl†) of BCIP/NBT Reagent 2
- Add 2 drops (≈ 80 μl†) of BCIP/NBT Reagent 3

Mix well before use. Use immediately.

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

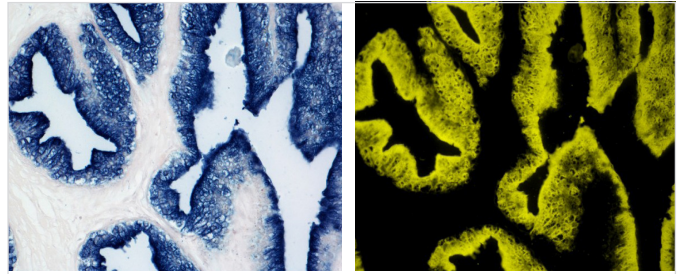
† Drop volumes differ due to solvent compositions.

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.

Instructions for Use

After incubation with an AP detection system, rinse sections with buffer. 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.



Prostate: Prostate Specific Antigen (PSA, m) detected with VECTASTAIN® ABC-AP Kit and BCIP/NBT Substrate (indigo), viewed under bright field microscopy (left) and viewed under fluorescence microscopy** (right).

For permanent, non-aqueous mounting: Dehydrate, clear, and coverslip using a non-aqueous mounting media, such as VectaMount® Mounting Medium (H-5000) or VectaMount® Express Mounting Medium (H-5700). BCIP/NBT is not compatible with DPX mountant.

For aqueous mounting: Coverslip using an aqueous mounting media such as VectaMount® AQ Mounting Medium (H-5501).

**Biotechniques (2007) 42: 756-759 and Biotechniques (2014) 57:254-256.

Detailed product listings, specifications, protocols and additional information is available on our website: vectorlabs.com