

BCIP/NBT

CHROMOGENIC SUBSTRATES

Precipitating AP Substrate

Cat. no. 4410

Product Characteristics

BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/ Nitro Blue Tetrazolium) liquid, ready-to-use substrate is a highly active and stable formulation utilized for colorimetric detection of Alkaline Phosphatase (AP) activity in membrane assays. Positive reactions form an intense blue/purple precipitate at the site of the reaction. The color develops when AP catalyzes the dephosphorylation of BCIP and converts NBT to insoluble blue/purple NBT formazan. The intense blue/purple precipitate is very stable and resists fading when exposed to light.

Composition & Properties

The BCIP/NBT is a liquid, ready-to-use substrate. The solution contains NBT, BCIP and non-toxic stabilizers in an aqueous buffer, pH 9.6. The formulation is free of organic solvents.

Working Procedure

1. Allow the solution to reach room temperature prior to use.
2. After the final incubation with the AP-labelled probe, wash the membrane thoroughly in a Tris-Buffered Saline (TBS Buffer) containing 0.1% Tween 20.
3. After the final wash, completely cover the membrane with BCIP/NBT solution and allow the color reaction to develop until optimal signal intensity is reached (usually 5-30 minutes). Incubation time will depend on enzyme activity. A further adjustment of the dilution of the AP probe may be necessary. The formazan deposit may flake off the membrane or background staining of the membrane may occur if the color development is too fast or too slow, respectively.
4. Stop the enzyme reaction by thoroughly washing membrane with deionized water.
5. Dry the membrane and store at room temperature. It is recommended to protect the membrane from light for a permanent record of results.

Tips & Tricks

- Normal appearance of the solution is clear, pale yellow. Discard if solution is turbid or purple. Crystalline precipitate can occur in the product.
- Do not use phosphate buffers, as inorganic phosphate is a powerful inhibitor of AP.
- The products are not recommended for immunohistochemical or in situ hybridization techniques.

Handling & Storage

- Store solution at 2-8 °C in the dark.
- Avoid exposure to light and heat.
- Re-dispense only into bottles made of High Density Polyethylene (HDPE), amber color. Dispensing guidelines are available upon request.