BCIP/NBT Alkaline Phosphatase (AP) Substrate Kit

Catalog number: 11948
Unit size: 500 tests

Component	Storage	Amount (Cat No. 11948)
Component A: BCIP Solution	Freeze (< -15 °C), Minimize light exposure	1 vial (500 µL)
Component B: NBT Solution	Freeze (< -15 °C), Minimize light exposure	1 vial (500 µL)
Component C: BCIP-NBT Staining Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (50 mL)

OVERVIEW

BCIP/NBT Alkaline Phosphatase (AP) Substrate Kit is a chromogenic detection system optimized for visualizing alkaline phosphatase (AP) activity in immunohistochemistry (IHC), in situ hybridization (ISH), and blotting applications. Upon enzymatic reaction with AP, the combined BCIP and NBT substrates generate an insoluble purple precipitate that is easily visualized under white light microscopy. This kit includes separate BCIP, NBT, and staining buffer components to prepare a working solution fresh before use. The system accommodates various staining needs, providing reliable detection in both tissue-based and membrane-based assays.

AT A GLANCE

1. Apply the BCIP-NBT working solution to the tissue sections
2. Incubate the tissue sections for 30 to 60 minutes
3. Rinse the tissue sections for 5 to 10 minutes
4. Apply the mounting medium to cover the section

Note: Before use, bring all the kit components to room temperature and centrifuge briefly to collect at the bottom.

KEY PARAMETERS

Light microscope

PREPARATION OF WORKING SOLUTION

Prepare BCIP-NBT Substrate working solution by adding 10 µL of BCIP solution (Component A), 10 µL of NBT solution (Component B), into 1 mL of BCIP-NBT Staining Buffer (Component C). Protect the working solution from light by covering it with foil or placing it in the dark.

Note: For best results, this solution should be used within 1 or 2 hours of its preparation.

Note: 10 mL of working solution is enough for 100 tests.

SAMPLE EXPERIMENTAL PROTOCOL

1. After incubation with Alkaline Phosphatase (AP) detection system, rinse tissue sections with buffer, i.e TBS buffer.
2. Add enough volume of BCIP-NBT Substrate working solution (100 µL) to cover the tissue section and incubate for 30 to 60 minutes in the dark at room temperature.
   Note: Optimal development times should be determined by the end user. The incubation time of this substrate can be lengthened (up to 24 hours) providing significantly more sensitivity.
3. Wash slides in TBS buffer or water for 5 minutes.
4. For the permanent, non-aqueous mounting, dehydrate, clear and coverslip using non-aqueous mounting medium. For aqueous mounting medium, coverslip using an aqueous mounting medium such as FluoroQuest™ PLUS Antifade Mounting Medium (Cat# 20008).
5. Use the bright field to visualize the stain.

EXAMPLE DATA ANALYSIS AND FIGURES

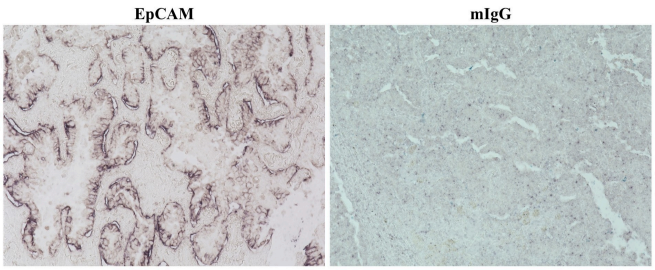

Figure 1. Immunohistochemical detection of EpCAM in FFPE lung adenocarcinoma tissue. The tissue sections were incubated with EpCAM or Mouse IgG primary antibodies followed by ALP conjugated Goat anti-Mouse IgG and then developed with BCIP/NBT Alkaline Phosphatase (AP) substrate kit.

DISCLAIMER

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