

Product Information

Alkaline Phosphatase Blue Microwell Substrate

Sufficient for 400 mL working substrate

AB0100

Product Description

5-Bromo-4-chloro-3-indolyl phosphate (BCIP®) and nitro blue tetrazolium (NBT) are reagents that are widely used in tandem for colorimetric detection of alkaline phosphatase-labeled molecules.¹ This product is supplied as a two-component buffered alkaline phosphatase substrate whose components each contain (a) a BCIP® analog and (b) NBT. Prior to reaction with alkaline phosphatase:

- The BCIP analog is a colorless to faint blue solution.
- The NBT reagent is a yellow solution.

The two-component mixture develops a bluish-purple product when reacted with alkaline phosphatase in microwell-type assays. This substrate is not recommended for membrane or immunohistochemical type assays that require an insoluble reaction product.

Components

BCIP® analog (Component A0227): 2 × 100 mL

NBT reagent (Component A9851): 2 × 100 mL

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

This product does not contain aprotic solvents. This reduces the environmental concerns associated with shipping and handling of the material.

Storage/Stability

Store the product at 2-8 °C. This substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Preparation Instructions

1. Prior to use, both solutions should be brought to room temperature (~25 °C).
2. Then mix equal volumes, of the researcher's choice, of the two component solutions.
3. The substrate mixture should be used within one hour.

Usage

This two-component Blue Microwell Substrate Solution is very sensitive. Great care must be taken to minimize background reactivity associated with nonspecific immunochemical reactivities.

To reduce the intensity of a reaction, dilution of the substrate is **not** recommended. It is recommended to dilute the utilized antibodies or conjugates instead.

- In each microwell containing alkaline phosphatase, add 200 µL of the substrate mixture. Following the reaction, a bluish-purple product forms.
- In kinetic assays, the reaction product should be read at a wavelength between 490 nm–650 nm.
- Endpoint assays produce a blue reaction product after the addition of 50 µL of Alkaline Phosphatase Stop Solution (Cat. No. A5852). Absorbance values of the reaction should be monitored and read before an absorbance value of 2.0 is attained.

References

1. Smejkal, G.B., and Kaul, C.A., *J. Histochem. Cytochem.*, **49(9)**, 1189-1190 (2001).

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