

BCIP/NBT 1-Component AP Membrane Substrate

A one-component formulation suitable for membrane assays using AP.

BCIP/NBT 1-Component AP Membrane Substrate is suitable for membrane applications using alkaline phosphatase (AP) as the conjugated detection enzyme. BCIP/NBT 1-Component AP Membrane Substrate should not be used for microwell (ELISA) applications.

BCIP/NBT 1-Component AP Membrane Substrate is a one-component formulation containing 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT) substrate. BCIP/NBT 1-Component AP Membrane Substrate reacts with alkaline phosphatase to yield an insoluble, dark blue reaction product. BCIP/NBT 1-Component AP Membrane Substrate is supplied ready-to-use at 1X.

Allow BCIP/NBT 1-Component AP Membrane Substrate to equilibrate to room temperature (25°C) prior to use. After probing with the antibody and AP reagents, wash membrane thoroughly and transfer the membrane into a clean container. Cover the membrane surface with ample amount of BCIP/NBT 1-Component AP Membrane Substrate and incubate. The substrate will react with sites on the membrane containing alkaline phosphatase, producing an insoluble, permanent, dark blue reaction product.

For best results, monitor the substrate color development process until the target protein bands are visible. To stop the reaction, rinse the membrane with reagent quality water. If the reaction proceeds too long, there will be excessive background staining and diminished resolution of the target peptide or protein banding regions. If the color development is too rapid or intense, it is recommended to dilute the antibodies or conjugates or shorten the incubation period.

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ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

BCIP/NBT 1-COMPONENT AP MEMBRANE SUBSTRATE

Size	Catalog#
100 mL	6281

INSTRUCTIONS:

1. Perform electro-blotting procedure.
2. Block membranes 4 hours-overnight.
3. Probe membranes with antibodies and AP conjugate.
4. Wash membranes after each antibody incubation step. Always transfer to a clean container for substrate development step.
5. Bring BCIP/NBT 1-Component AP Membrane Substrate to room temperature; protect it from light.
6. Add BCIP/NBT 1-Component AP Membrane Substrate to cover the membrane surface.
7. Incubate BCIP/NBT 1-Component AP Membrane Substrate at room temperature for several minutes.
8. Monitor the substrate color development to visualize the target peptide and protein bands.
9. Stop the color development reaction by transferring the membrane to diH₂O. Change the solution several times to ensure complete removal of all soluble BCIP/NBT components.
10. Analyze the data.

For more information and protocols, please visit www.immunochemistry.com.

SPECIFICATIONS:

- Colorless to very light yellow liquid
- 1X ready to use

STORAGE:

- 2-25°C
- Protect from light

SAFETY & USAGE:

- SDS available at immunochemistry.com
- Not for human or drug use
- For research use only

