Tribioscience

For research use only

NCIP/NBT Substrate System (Catalog# TBS5022)

For colorimetric AP-based Blot & IHC detection

Description

The 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) Substrate System is an ideal insoluble substrate for use with alkaline phosphatase. This system produces a blue-purple product. The intense color can be observed visually, is very stable, and will not fade upon exposure to light. The BCIP/NBT Substrate System offers a convenient, ready-to-use substrate solution for visualizing alkaline phosphatase activity in immunoblotting and immunohistology. Immunohistochemical staining with BCIP/NBT requires organic mounting media and can be counterstained with Nuclear Fast Red or Light Green. BCIP/NBT is generally a more sensitive method than Fast Red.

Application:

BCIP/NBT Solution is formulated to precipitate and localize the blue-purple reaction product onto membrane surfaces or tissue samples at sites where the alkaline phosphatase-substrate reaction occurs. BCIP-NBT Substrate produces a very large signal-to-noise ratio, although its high sensitivity requires careful washing and blocking to avoid background staining. When performing immunohistochemical techniques, organic mounting media must be used.

Highlights:

- **AP substrate** – for detection of alkaline phosphatase (alk-phos) activity on solid media, including nitrocellulose and PVDF membranes and fixed tissue samples
- **Chromogenic** – no special equipment needed for visualization; produces purple-black precipitate that is easy to photograph

Storage: Supplied as a ready to use solution, 2x 125 mL (TBS5022-IHC & TBS5022-Blot). Store at 2-8°C for up to one year.

Procedure:

Western blotting

1. Remove blot from the transfer apparatus and block nonspecific sites with Blocking Buffer for 10-30minutes at room temperature with shaking.
2. Add the primary antibody and incubate membrane for 1 hour with shaking.
3. Wash the membrane with TBS-T.
4. Add the HRP-conjugated secondary antibody and incubate membrane for 1 hour at room temperature with shaking.
5. Wash membrane with TBS-T.
6. Add the BCIP/NBT Substrate solution to the membrane and carefully monitor color development.
7. Stop the reaction by rinsing membrane with water.

Immunohistochemistry

Rinse specimens incubated with an alkaline phosphatase conjugate in a non-phosphate wash buffer before treatment with the BCIP/NBT Substrate System. Cover the entire specimen with the reagent during color development. Incubate the specimen at room temperature with the BCIP/NBT reagent for ~10 minutes. Specimens and procedure may affect the length of time needed for color development. Monitor color development to avoid over development. Stop color development by rinsing the specimen with water.

RELATED PRODUCTS:

TBST-10x (catalog# TBS5008)
BSA Standard solution (catalog# TBS5002)
Protein Assay kit (catalog# TBS2005)
Cell Lysis Buffer (10x) (catalog# TBS5001)
RIPA Buffer (catalog# TBS5017)
Antibody Diluent Kit (catalog# TBS5012)

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Material Safety Data Sheet (MSDS)

Identification:

Product name: NCIP/NBT Substrate System
Product Catalog: TBS5022
Manufacturer Supplier:
4062 Fabian Way, suite 1, Palo Alto,
CA 94303 USA
1-650-917-9269 TEL
1-650-618-5498 FAX

Composition/Information on Ingredients:

This product is For Research Use Only. According to 29 CFR 1910.1200(d), mixtures with hazardous ingredients at less than <1% and carcinogens at less than < 0.1% are considered non-hazardous.

Ingredient Name	CAS#	Percent
5-bromo-4-chloro-3-indolyl-phosphate (NCIP)	6578-06-9	<1%
nitro blue tetrazolium (NBT)	298-83-9	<1%
2-propanol	127-19-5	<1%

Hazard Identification:

CAUTION: This product is not for use in humans. It is intended for research purposes only. To the best of our knowledge, the chemical, physical, and toxicological properties of this material have not been established.
Emergency Overview: Harmful by Ingestion. Irritant.
Potential Health Effects:
Inhalation: May be harmful if inhaled. Causes respiratory tract irritation.
Eye Contact: Causes eye irritation. Risk of damage to eyes.
Skin Contact: May be harmful if absorbed through skin. Causes skin irritation.
Ingestion: Harmful if swallowed.

First Aid Measures for hazardous ingredient:
2-propanol:
Inhalation: Remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: DO NOT INDUCE VOMITING. If person is conscious, wash out mouth with water. Get medical attention.
Skin contact: Wash skin with soap or mild detergent and water for at least 15 minutes. If irritation develops or persists, get medical attention.
Eye contact: Immediately flush eyes water for at least 15 minutes. Get medical attention.

Accidental Release Measures:

Wear appropriate personal protective equipment as indicated. Absorb liquid with an absorbent material. Transfer contaminated absorbent to a closed chemical waste container for disposal. Wash spill site after material has been picked up for disposal.

Handling and Storage:

Store at 4°C in tightly closed container. Avoid inhalation of vapor or mist. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. Avoid prolonged or repeated exposure.

Exposure Controls/Personal
Ventilation System: a system of local (fume hood) and general exhaust is recommended.
Skin Protection: wear compatible chemical resistant gloves and protective clothing.
Eye protection: wear chemical safety goggles. Maintain emergency eyewash and shower in work area.

Physical and Chemical Properties
Appearance: colorless liquid **Odor:** data not available
pH: data not available
Boiling Point: data not available
Melting or Freezing Point: data not available
Flash Point: data not available
Volatile Organic Compounds: data not available
Autoignition temp: data not available
Solubility (water): Soluble in water

Toxicological Information: Acute toxicity: data not available **Chronic exposure:** data not available
Potential Health Effects:
Inhalation: May be harmful if inhaled. Causes respiratory tract irritation.
Skin: May be harmful if absorbed through skin. Causes skin irritation.
Eyes: Causes eye irritation.
Ingestion: Harmful if swallowed.

Disposal Considerations:

Dispose of in accordance with federal, state and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

Other Information:

This product is for research use only and is not intended for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. The above information is believed to be accurate but is not necessarily all-inclusive and shall be used only as a guide. Tribioscience, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.