For life science research only.
Not for use in diagnostic procedures.

NBT/BCIP Stock Solution

Version: 09
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Cat. No. 11 681 451 001 8 ml

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	NBT/BCIP Stock Solution	▪ Solution of 18.75 mg/ml nitro blue tetrazolium chloride and 9.4 mg/ml 5-bromo-4-chloro-3-indolyl-phosphate, toluidine-salt in 67% DMSO (v/v). ▪ The color of the solution can vary between yellow to light brown.	1 bottle, 8 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	NBT/BCIP Stock Solution	Store at +2 to +8°C. Alternatively, store at least 4 weeks at +15 to +25°C. ⚠ ***Keep protected from light.*** ⚠ ***If precipitates occur in the color substrate solution, warm the solution to +50°C. If the precipitates remain, centrifuge the tube prior to use as the precipitates may cause background; centrifugation will not reduce the overall sensitivity.***

1.3. Additional Equipment and Reagent required

For immunodetection of digoxigenin-labeled biomolecules

- DIG-High Prime DNA Labeling and Detection Starter Kit I*
- DIG DNA Labeling and Detection Kit*
- DIG Nucleic Acid Detection Kit*

For immunodetection of biotin-labeled glycoconjugates and proteins

i *See section,* **Working Solution** *for information on preparing solutions.*

- TBS: 0.05 M Tris-HCl*, 0.15 M NaCl, pH 7.5
- Staining solution
- Streptavidin-AP-conjugate*
- Blocking Reagent*
- Tween 20*
- Microwave oven
- $MgCl_2$

1.4. Application

The NBT/BCIP solution is used for the sensitive detection of alkaline phosphatase (AP). Both dyes have very little solubility in water or lipid and can be used for:

- AP-detection in immunoblotting.
- Immunohistochemical assays

2. How to Use this Product

2.1. Before you Begin

Working Solution

Preparation of staining solution

Application	Preparation/Composition	Storage and Stability
DIG system	Add 200 µl NBT/BCIP Stock Solution to 10 ml 0.1 M Tris-HCl*, pH 9.5 (+20°C), 0.1 M NaCl. ⚠ ***Do not include $MgCl_2$ in the DIG detection buffer as this might lead to spotty background on the membrane after the detection procedure.***	Always prepare fresh.
All other applications	Add 200 µl NBT/BCIP Stock Solution to 10 ml 0.1 M Tris-HCl, pH 9.5 (+20°C), 0.1 M NaCl, 0.05 M $MgCl_2$.	

For immunodetection of biotin-labeled glycoconjugates

Solution	Preparation/Composition	Storage and Stability	For use in...
TBS	0.05 Tris-HCl*, 0.15 M NaCl, pH 7.5.	Store at +15 to +25°C.	Preparation of Blocking solution.
Blocking solution	Dissolve 0.5 g Blocking Reagent* in 100 ml TBS, pH 7.5 by heating to +50 to +60°C for 1 hour. The dissolution can be accelerated by sonication or by incubation in a microwave oven. ⓘ *The solution remains turbid.*	Store at +2 to +8°C.	Blocking of nonspecific binding sites on the membrane.
Antibody solution	▪ Add 5 µl of Streptavidin-AP* conjugate to 10 ml TBS, Tween 20*, 0.1% (w/v). ▪ Centrifuge the antibody for 5 minutes at 10,000 rpm in the original vial prior to each use, and pipette the necessary amount carefully from the surface.	Store 12 hours at +2 to +8°C.	Binding to the biotin-labeled probe.

2.2. Protocols

Immunodetection of digoxigenin-labeled biomolecules

ⓘ *See section,* **Working Solution** *for information on preparing solutions.*
The Staining solution is used for the detection of nucleic acids, proteins, and glycoconjugates. Refer to the Instructions for Use of the following kits:
- DIG-High Prime DNA Labeling and Detection Starter Kit I*
- DIG DNA Labeling and Detection Kit*
- DIG Nucleic Acid Detection Kit*

⚠ ***The Staining solution can substitute for the individual staining solutions in the kits.***

Immunodetection of biotin-labeled glycoconjugates and proteins

The volumes stated refer to a 50 to 100 cm^2 filter.

⚠ ***Incubate all filters by gentle agitation at +15 to +25°C except for color development which is done without shaking.***

See section, **Working Solution** *for information on preparing solutions.*

1. Incubate the filter with the immobilized biotin-labeled samples for at least 30 minutes in approximately 20 ml Blocking solution.

   If necessary, the detection can be interrupted at this stage and the filter kept in the Blocking solution at +2 to +8°C.

2. Wash 3 × 10 minutes with approximately 50 ml TBS.

3. Incubate the filter with 10 ml Antibody solution for 1 hour.

4. Wash 3 × 10 minutes with approximately 50 ml TBS.

5. Immerse the filter without agitation in the freshly prepared Color substrate solution and observe the development of the blue color.

   The color reaction is normally finished within minutes but can take up to one hour or even overnight if very little sample is present. The detection depends highly on the nature of the biotin-labeled sample.

6. Rinse the filter several times with double-distilled water to stop staining.
   – Dry the filter on paper towels; the filter can now be photographed or photocopied directly.

   Filters can be stored protected from light for documentation.

In situ hybridization

⚠ ***For nonradioactive in situ hybridization with alkaline phosphatase and the NBT/BCIP chromogen, do not use xylene-based mounting media. This can cause crystal formations in the color precipitates.***

2.3. Parameters

Chemical Formula

NBT (Nitro blue tetrazolium chloride): $C_{40}H_{30}Cl_2N_{10}O_6$
BCIP (5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt): $C_8H_6NO_4BrClP \times C_7H_9N$

Chemical Name

Chemical structure NBT

NBT: Nitro blue tetrazolium chloride

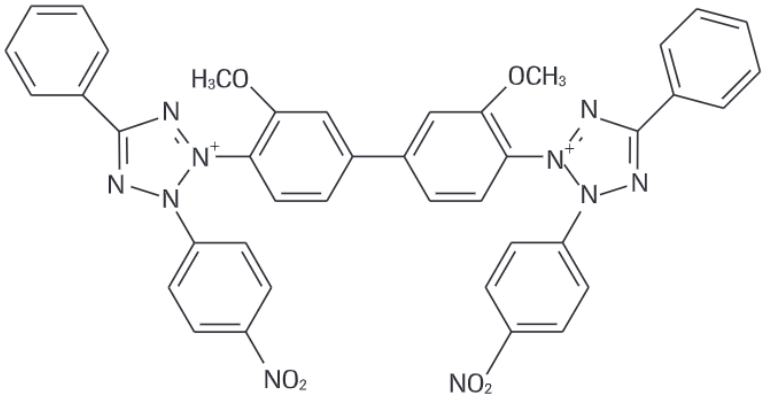

Fig. 1: Chemical structure of NBT.

Chemical structure BCIP

BCIP: 5-bromo-4-chloro-3-indolyl phosphate

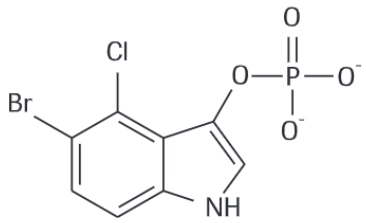

Fig. 2: Chemical structure of BCIP.

Molecular Weight

NBT: 817.7 g/mol
BCIP: toluidine salt: 433.6 g/mol

3. Additional Information on this Product

3.1. Test Principle

Reaction principle

① BCIP is the alkaline phosphatase (AP) substrate, which after dephosphorylation, is oxidized by NBT to yield a dark-blue indigo precipitating dye. NBT is thereby reduced to a dark-blue precipitating dye and serves to intensify the color reaction making the detection more sensitive (Figure 3).

② Both dye reaction products have little solubility in water or lipid and can be used for AP detection in immunoblotting and immunohistochemical assays.

③ The reaction proceeds at a steady rate, allowing accurate control of the relative sensitivity and control of the development of the reaction.

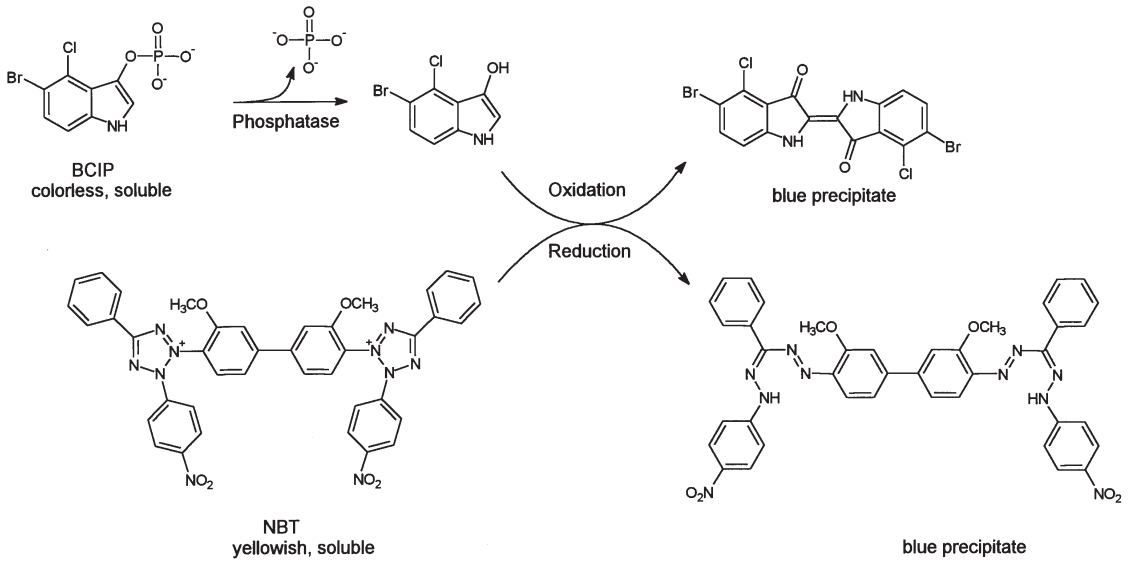

Fig. 3: Mechanism for the dye-generating redox reaction.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
DIG Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910
DIG-High Prime DNA Labeling and Detection Starter Kit I	1 kit, 12 labeling reactions of 10 ng to 3 µg DNA and detection of 24 blots of 100 cm^2	11 745 832 910
DIG DNA Labeling and Detection Kit	1 kit, 25 labeling reactions of 10 ng - 3 µg DNA and detection of 50 blots of 100 cm^2	11 093 657 910
Blocking Reagent	27 g, for one liter blocking solution, *Not available in US*	11 112 589 001
Tris hydrochloride	500 g	10 812 846 001
Streptavidin Conjugates	Streptavidin-AP Conjugate, 1,000 U	11 089 161 001
	Streptavidin-POD Conjugate, 500 U	11 089 153 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

1121.11682318001 ®

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