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

NBT/BCIP Ready-to-Use Tablets

Version: 08
Content Version: November 2021

Cat. No. 11 697 471 001 20 tablets

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	NBT/BCIP Tablets	▪ Ready-to-use tablets. ▪ Each tablet contains substrates and buffer components for 10 ml staining solution.	1 bottle, 20 tablets

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 Tablets	Store dry at +2 to +8°C. ⚠ ***Keep protected from light.***

1.3. Additional Equipment and Reagent required

For immunodetection of digoxigenin-labeled biomolecules

- DIG Wash and Block Buffer Set*
- Anti-Digoxigenin-AP, Fab fragments*
- Double-distilled water
- TE buffer: 10 mM Tris-HCl*, 1 mM EDTA, pH 8.0 (+20°C)
- Nylon Membranes*
- Hybridization Bags

For immunodetection of biotin-labeled glycoconjugates and proteins

- Streptavidin-AP*
- Tween 20*
- Blocking Reagent*
- TBS: 0.05 M Tris-HCl*, 0.15 M NaCl, pH 7.5
- TE buffer: 10 mM Tris-HCl*, 1 mM EDTA, pH 8.0 (+20°C)
- Double-distilled water
- Nylon Membranes*
- Hybridization Bags

1.4. Application

The NBT/BCIP solution is used for a variety of applications:

- Sensitive detection of alkaline phosphatase in blotting protocols, such as Southern, northern, and western blotting, and colony and plaque lifts.
- Immunohistochemistry and immunocytochemistry.
- Detection of nucleic acids, proteins, and glycoconjugates.

i *NBT/BCIP may be used with both nitrocellulose and nylon membranes*.*

2. How to Use this Product

2.1. Before you Begin

Working Solution

Preparation of color substrate solution

Solution	Composition/Preparation	Storage and Stability	For use in...
Color substrate solution	Dissolve 1 NBT/BCIP Tablet in 10 ml double-distilled water to prepare a ready-to-use staining solution. Composition of staining solution after addition of double-distilled water: 0.4 mg/ml NBT, 0.19 mg/ml BCIP, 100 mM Tris buffer, pH 9.5, 50 mM $MgSO_4$. *The color of the solution can vary from slightly yellowish-violet to light brown.*	⚠ ***Prepare shortly before use.***	Preparation of color substrate solution.

For immunodetection of digoxigenin-labeled biomolecules

Solution	Composition/Preparation	Storage and Stability	For use in...
Washing buffer	0.1 M maleic acid, 0.15 M NaCl, pH 7.5 (+15 to +25°C), 0.3% (v/v) Tween 20*	Store at +15 to +25°C.	Removal of unbound antibody.
Maleic acid buffer	0.1 M maleic acid, 0.15 M NaCl; adjust with NaOH (solid) to pH 7.5 (+15 to +25°C)		Dilution of Blocking solution.
Detection buffer	0.1 M Tris-HCl*, 0.1 M NaCl, pH 9.5 (+15 to +25°C)		Adjustment of pH to 9.5.
Blocking stock solution, 10x conc.	Dissolve Blocking Reagent* 10% (w/v) in Maleic acid buffer under constant stirring on a heating block (+65°C), or heat in a microwave oven and autoclave. *The solution remains opaque.*	Store at +2 to +8°C initially; after first usage, store in aliquots at −15 to −25°C.	Preparation of Blocking solution.
Blocking solution, 1x conc.	Dilute the 10x Blocking solution 1:10 in Maleic acid buffer.	Always prepare fresh.	Blocking of nonspecific binding sites on the membrane.
Antibody solution	▪ 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. ▪ Dilute Anti-Digoxigenin-AP, Fab fragments* 1:10,000 (75 mU/ml) in Blocking solution.	Store at +2 to +8°C.	Binding to the DIG-labeled probe.
TE buffer	10 mM Tris-HCl*, 1 mM EDTA, pH 8.0 (+20°C)	Store at +15 to +25°C.	Stopping the color reaction.

The Washing buffer, Maleic acid buffer, Blocking solution, and Detection buffer are available DNase- and RNase-free in the DIG Wash and Block Buffer Set.*

For immunodetection of biotin-labeled glycoconjugates and proteins

Solution	Composition/Preparation	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.
TE buffer	10 mM Tris-HCl*, 1 mM EDTA, pH 8.0 (+20°C)	Store at +15 to +25°C.	Stopping the color reaction.

2.2. Protocols

For immunodetection of digoxigenin-labeled biomolecules

The volumes stated refer to a 100 cm² filter; adjust the volumes for other filter sizes.

⚠ ***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 additional information on preparing solutions.*

The following steps describe the detection of digoxigenin-labeled biomolecules after hybridization.

1. Wash filter briefly, approximately 1 minute in Washing buffer.

2. Incubate for 30 minutes in 100 ml Blocking solution.

3. Incubate filter for 30 minutes in 20 ml Antibody solution.

4. Wash 2 × 15 minutes in 100 ml Washing buffer to remove unbound antibody conjugate.

5. Equilibrate filter 2 to 5 minutes in 20 ml Detection buffer.

6. Incubate filter with approximately 10 ml freshly prepared Color substrate solution sealed in a plastic bag or in a suitable box in the dark.
   – The color precipitate starts to form within a few minutes and the action is usually finished after 16 hours.

   ⚠ ***Do not shake or mix while color is developing.***

7. When the desired spots or bands are detected, stop the reaction by washing the filter for 5 minutes with 50 ml TE-buffer or double-distilled water.
   – The results can be documented by photocopying the wet filter or by photography.

   Photocopying onto overhead transparencies allows for densitometric scanning. For this purpose, the color reaction can be interrupted for a short time and continued later.

For immunodetection of biotin-labeled glycoconjugates and proteins

The volumes stated refer to a 50 to 100 cm² 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 development 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 TE-buffer or 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.

2.3. Parameters

Chemical Formula

NBT: $C_{40}H_{30}Cl_2N_{10}O_6$
BCIP: $C_8H_6NO_4BrClP \times C_7H_9N$

Chemical Name

Chemical structure NBT

NBT: Nitro blue tetrazolium chloride

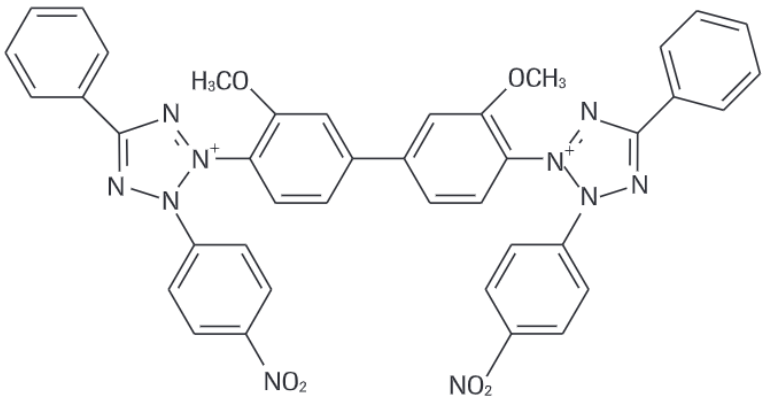

Fig. 1: Chemical structure of NBT.

Chemical structure BCIP

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

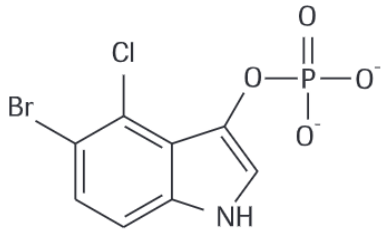

Fig. 2: Chemical structure of BCIP.

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.

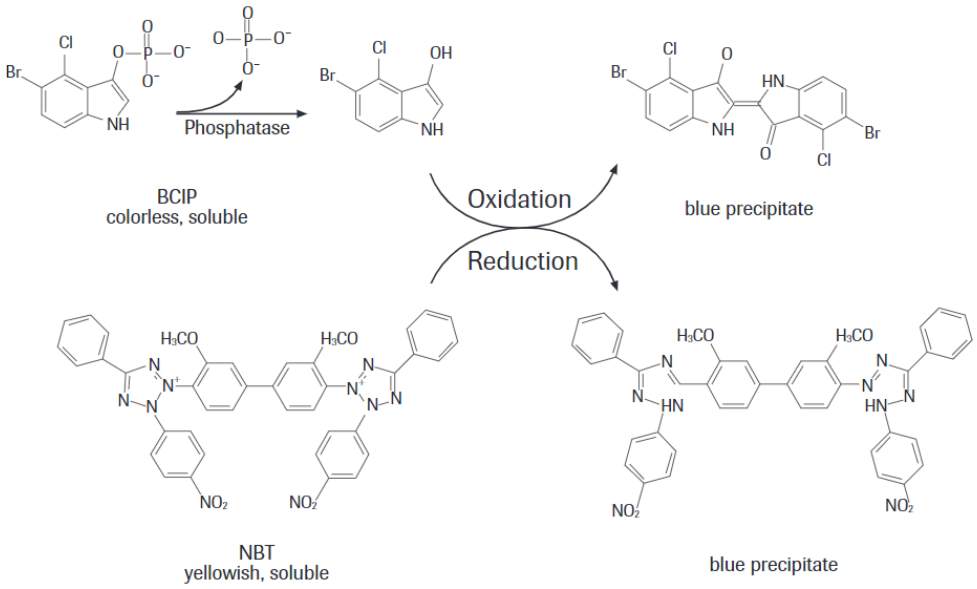

Fig. 3: Reaction mechanism of the dye-generating redox reaction.

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.
Consumables		
Hybridization Bags	50 bags, 25 cm x 23 cm	11 666 649 001
Reagents, kits		
Blocking Reagent	50 g	11 096 176 001
Tris hydrochloride	500 g	10 812 846 001
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
Streptavidin-AP-conjugate for nucleic acid detection	150 U, (200 µl)	11 093 266 910
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910
Nylon Membranes, positively charged	10 sheets, 20 x 30 cm	11 209 272 001
	20 sheets, 10 x 15 cm	11 209 299 001
	1 roll, 0.3 x 3 m	11 417 240 001
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

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