Print

NBT/BCIP Ready-to-Use Tablets

Cat. No. 11 697 471 001 20 tablets

Version 06
Content version: March 2007
Store at +2 to +8°C

1. What this Product Does

Contents

- 1 bottle with 20 ready-to-use tablets.
- Each tablet contains substrates and buffer components for 10 ml staining solution

Storage and Stability

Tablets are stable at +2 to +8 °C until to the expiration date printed on the bottle label.

⚠ Store dry and protected from light.

🔍 The product is shipped at ambient temperature.

Application

The solution is used for the sensitive detection of alkaline phosphatase in blotting protocols including:

- Southern blot
- northern blot
- western blot
- colony and plaque lifts

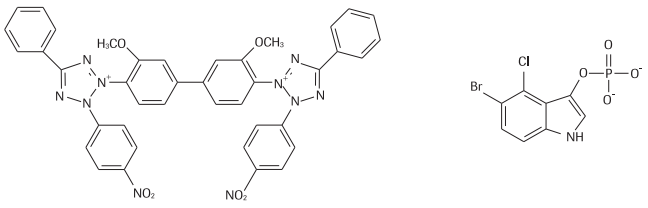
and for the application in immunohistochemistry and immunocytochemistry.

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

Product Characteristics

- **NBT**: Nitro blue tetrazolium chloride; $C_{40}H_{30}Cl_2N_{10}O_6$; MW =817.7
- **BCIP**: 5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt; $C_8H_6NO_4BrCIP \times C_7H_9N$; MW =433.6

Structure



NBT BCIP

Fig. 1: Chemical formula of NBT and BCIP

Reaction Principle

BCIP (1) is the alkaline phosphatase (AP) substrate, which after dephosphorylation is oxidized by NBT to yield a dark-blue indigo precipitating dye. NBT (2) is thereby reduced to a dark-blue precipitating dye and serves to intensify the color reaction making the detection more sensitive.

Both dye reaction products are very little soluble in water or lipid and can be applied for AP detection in immunoblotting (3) and immunohistochemical (4) assays.

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

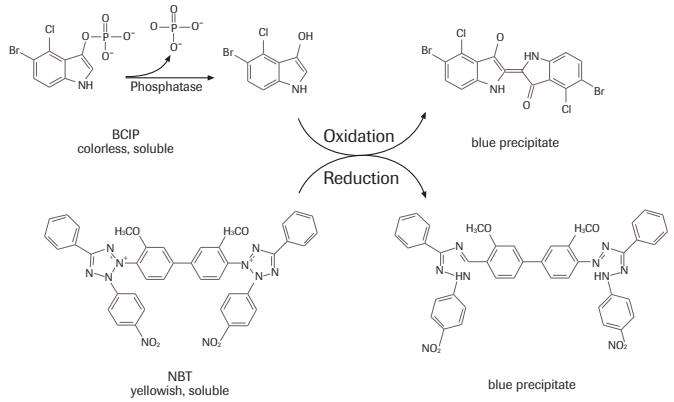


Fig. 2: Reaction scheme of the dye-generating redox reaction.

2. How to Use this Product

2.1 Preparation of Color Substrate Solution

Add 1 tablet to 10 ml double dist. water to prepare a ready-to-use staining solution.

Composition of staining solution (pH 9.5) after addition of double dist. 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 the solution shortly before use.

2.2 Immunodetection of Digoxigenin-labeled Biomolecules

Additional Application

The color substrate solution can generally be used for the detection of nucleic acids, proteins and glycoconjugates.

The staining solution can substitute the individual staining solutions in the kits listed below.

🔍 Please follow the instructions given in the respective kit's package insert.

Cat. No.	Kit
11 093 657 001	DIG DNA Labeling and Detection Kit
11 175 041 001	DIG Nucleic Acid Detection Kit
11 142 372 001	DIG Glycan Detection Kit
11 210 238 001	DIG Glycan Differentiation Kit

Additional Reagents Required

- DIG Wash and Block Buffer Set*
- Anti-digoxigenin-AP, Fab fragments*
- Double dist. water

Preparation of Additional Solutions Required

🔍 The Washing buffer, Maleic acid buffer, and Detection buffer are also available free of DNase and RNase, according to the current quality control procedures, in the DIG Wash and Block Buffer Set*.

Solution	Composition/Preparation	Storage/ Stability	Use
Washing buffer	0.1 M Maleic acid, 0.15 M NaCl; pH 7.5 (+15 to +25°C); 0.3% (v/v) Tween 20	+15 to +25°C, stable	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)	+15 to +25°C, stable	Dilution of Blocking solution
Detection buffer	0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5 (+15 to +25°C)	+15 to +25°C, stable	Adjustment of pH to 9.5
Blocking stock solution, 10× 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, autoclave. Q The solution remains opaque.	+2 to +8°C	Preparation of Blocking solution
Blocking solution	Prepare a 1× working solution by diluting the 10× Blocking solution 1:10 in Maleic acid buffer.	Always prepare fresh	Blocking of unspecific binding sites on the membrane
Antibody solution	Centrifuge the antibody for 5 min at 10,000 rpm in the original vial prior to each use, and pipet the necessary amount carefully from the surface. Dilute anti-digoxigenin-AP 1:10,000 (75 mU/ml) in Blocking solution.	+2 to +8°C	Binding to the DIG-labeled probe
TE-buffer	10 mM Tris-HCl, 1 mM EDTA, pH 8.0 (20 °C).	+15 to +25°C	Stopping the color reaction

Procedure

In the following table the detection of digoxigenin-labeled biomolecules after hybridization is described.

Q All incubations are performed at +15 to +25°C and except for the color reaction with shaking or mixing. The volumes of the solutions are calculated for a filter size of 100 cm^2 and should be adjusted to other filter sizes

❶	Wash filter briefly (1 min) in **Washing buffer**
❷	Incubate for 30 min in 100 ml **Blocking solution**.
❸	Incubate filter for 30 min in 20 ml **Antibody solution**.
❹	Wash 2 × 15 min in 100 ml **Washing buffer** to remove unbound antibody conjugate.
❺	Equilibrate filter 2–5 min in 20 ml **Detection buffer**.
❻	Incubate filter with ca. 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 complete after 16 h. Do not shake or mix while color is developing.
❼	When the desired spots or bands are detected, stop the reaction by washing the membrane for 5 min with 50 ml **TE-buffer** or **double distilled water**.

Documentation of Results

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 afterwards).

2.3 Immunodetection of Biotin-labeled Glycoconjugates and Proteins

Additional reagents required

- Strepatividin-AP*
- Tween 20*
- Blocking Reagent*
- Double dist. water

Preparation of Additional Solutions Required

Solution	Composition/Preparation	Storage/ Stability	Use
TBS	0.05 M Tris-HCl*, 0.15 M NaCl, pH 7.5	+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 - 60°C (1 h). The dissolution can be accelerated by sonication or by incubation in a microwave oven. Q The solution remains turbid.	+2 to +8°C, stable	Blocking of unspecific binding sites on the membrane
Antibody solution	Add 5 ml of Streptavidin-AP conjugate to 10 ml TBS, Tween 20*, 0.1 % (w/v). ⚠ Centrifuge the antibody for 5 min at 10,000 rpm in the original vial prior to use, and pipet the necessary amount carefully from the surface.	12h 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)	+15 to +25 °C	Stopping the color reaction

Procedure

Gently agitate the filter while incubating at +15 to +25°C, except for color development which should be performed without motion.

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

Step	Action
❶	Incubate the filter with the immobilized biotin-labeled samples for at least 30 min in approx. 20 ml Blocking solution. Q The procedure can be interrupted at this stage if necessary and the filter can be kept in the Blocking solution at +2 to +8°C.
❷	Wash 3 times for 10 min each with approx. 50 ml TBS.
❸	Incubate the filter with 10 ml Antibody solution for 1 h.
❹	Wash 3 times for 10 min each with approx. 50 ml TBS.
❺	Immerse the filter without agitation in the freshly prepared Color substrate solution and observe the development of the blue color. Q The development is normally complete within minutes but can take for 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. Rinse the filter several times with TE-buffer or double dist. water to stop staining. Dry the filter on paper towels. The filter can now be photographed or photocopied directly. Q Filters can be stored protected from light for documentation.

3. Additional Information on this Product

References

1 Horwitz, J. P. *et al.* (1966), Substrates for Cytochemical Demonstration of Enzyme Activity. II. Some Dihalo-3-indolyl Phosphates and Sulfates. *J. Med. Chem.* **9**, 447.

2 Michal, G. *et al.*: Chemical design of indicator reactions for the visible range. In: Methods of Enzymatic Analysis (Bergmeyer, H. U. ed.) 3rd edition, 1983, Vol I, 197.

3 Wolf, P. L. *et al.* (1968), A new histochemical stain for neutrophilic leukocyte alkaline phosphatase. *Enzymologia (Enzyas)* **35**, 154 - 156.

4 Wolf, P. L. *et al.* (1973): A Test for Bacterial Alkaline Phosphatase: Use in Rapid Identification of *Serratia* Organisms. *Clin. Chem.* **19**, 1248-49.

4. Supplementary Information

4.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Usage
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Changes to previous version

Regulatory Disclaimer updated

4.2 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Sites including:

- DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection: http://www.roche-applied-science.com/dig

Product	Pack Size	Cat No.
DIG DNA Labeling and Detection Kit	25 labeling reactions and 50 blots , 10 × 10 cm	11 093 657 910
DIG Nucleic Acid Detection Kit	40 blots, 10×10 cm	11 175 041 910
DIG Glycan Differentiation Kit	5 × 5 blots, 10×10 cm	11 210 238 001
Tris-HCl	500 g	10 812 846 001
Blocking Reagent	50 g	11 096 176 001
DIG Wash and Block Buffer Set	30 blots, 10×10 cm	11 585 762 001
Streptavidin-AP	150 U 100 U (1 ml)	11 093 266 910 11 089 161 001
Hybridization Bags	50 bags	11 666 649 001
Anti-Digoxigenin-AP, Fab Fragments	150 U (250 µl) 100 sheets, 18×24 cm	11 093 274 910
Nylon Membrane, positively charged	10 sheets, 20×30 cm 20 sheets, 10×15 cm 1 roll, 0.3×3 m[#]	11 209 272 001 11 209 299 001 11 417 240 001
Tween 20	5 × 10 ml	11 332 465 001

[#] *not available in the US*

4.3 Trademarks

Regulatory Disclaimer

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

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