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

4-Nitro blue tetrazolium chloride (NBT)

Version: 20
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Solution

Cat. No. 11 383 213 001	3 ml 300 mg

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	4-Nitro blue tetrazolium chloride (NBT)	▪ 100 mg/ml solution in 70% dimethylformamide (DMF) (v/v). ▪ The color of the NBT solution can vary between yellow and brown depending on the lot used. ⚠ ***The color does not impair the quality or the function of the reagent. If a precipitate occurs, warm the solution briefly at +37°C.***	1 vial, 3 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	4-Nitro blue tetrazolium chloride (NBT)	Store at −15 to −25°C. ⚠ ***Keep protected from light.***

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*
- BCIP*
- Blocking Reagent*
- Tween 20*
- Microwave oven
- $MgCl_2$

1.4. Application

NBT is used as a redox indicator in combination with BCIP for the sensitive detection of alkaline phosphatase (AP). Both dyes have very little solubility in water or lipid and can be applied for the AP detection in:

- Immunoblotting
- Immunohistochemical assays

2. How to Use this Product

2.1. Before you Begin

Safety Information

4-Nitro blue tetrazolium chloride contains dimethylformamide and is toxic.
⚠ ***The product may cause harm to the unborn child; avoid exposure.***

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Preparation of staining solution		
Application	**Preparation/Composition**	**Storage and Stability**
DIG system	Add 50 µl NBT solution and 37.5 µl BCIP 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. Alkaline phosphatase does not require Mg^{2+}.***	⚠ ***Always prepare fresh.***
All other applications	Add 50 µl NBT solution and 37.5 µl BCIP to 10 ml 0.1 M Tris-HCl, pH 9.5 (+20°C), 0.1 M NaCl, 0.05 M $MgCl_2$.	
Preparation of blocking solution	**Preparation/Composition**	
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 ultrasonication or by incubation in a microwave oven. ⓘ *The solution remains turbid.*	–

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 times for 10 minutes each with approximately 50 ml TBS.

3. Add 5 µl of the Streptavidin-AP conjugate* to 10 ml TBS, 0.1% Tween 20* (w/v); incubate the filter in this solution for 1 hour.

4. Wash 3 times for 10 minutes each with approximately 50 ml TBS.

5. Immerse the filter without shaking in the Staining solution and observe the development of the blue color.
   The color reaction is normally completed within a few minutes, but can take up to one hour or overnight if very little sample is present. The detection limit depends greatly on the type of the biotin-labeled sample.

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

$C_{40}H_{30}Cl_2N_{10}O_6$

Molecular Weight

817.7 g/mol

3. Additional Information on this Product

3.1. Test Principle

How this product works

- NBT is a potent redox indicator forming an insoluble diformazane upon reduction (E′ = + 50 mV). The preparation is especially used if the dye developed should be stable for a prolonged time or if used for histochemical purposes; the equilibrium of the dehydrogenase reaction makes it necessary to remove the reduced product effectively from the reaction mixture. The sensitivity compared to the UV-determination of NAD(P)H at 339 nm is increased by approximately factor 2.
- As electron donors, for example, NADH or NADPH can be used. In this case, an intermediate hydrogen carrier (enzymes of the respiratory chain, diaphorase, or synthetic compounds such as 5-methyl phenazonium methyl sulphate [PMS]) is applied. PMS is autoxidable. The reduction of tetrazoliumchloride in competition with O_2 is favored in the presence of a detergent, such as Triton X-100* or Tween 80 in a 3-fold CMC-concentration.
- NBT is especially used as electron acceptor for the oxidation of 5-bromo-4-chloro-3-indoxyl. The product of dephosphorylation derived from 5-bromo-4-chloro-3- indolyl phosphate* (BCIP). This substrate is used in the sensitive detection of linked alkaline phosphatase, for example, after blotting procedures on appropriate supports, such as nylon membranes or nitrocellulose for the detection of nucleic acids, proteins, or glycoconjugates.

Reaction mechanism

① BCIP is the AP substrate which reacts further after the dephosphorylation to give a dark-blue indigo dye as an oxidation product.

② NBT serves as the oxidant and gives also a dark-blue dye. It intensifies the color and makes the detection more sensitive (Figure 1).

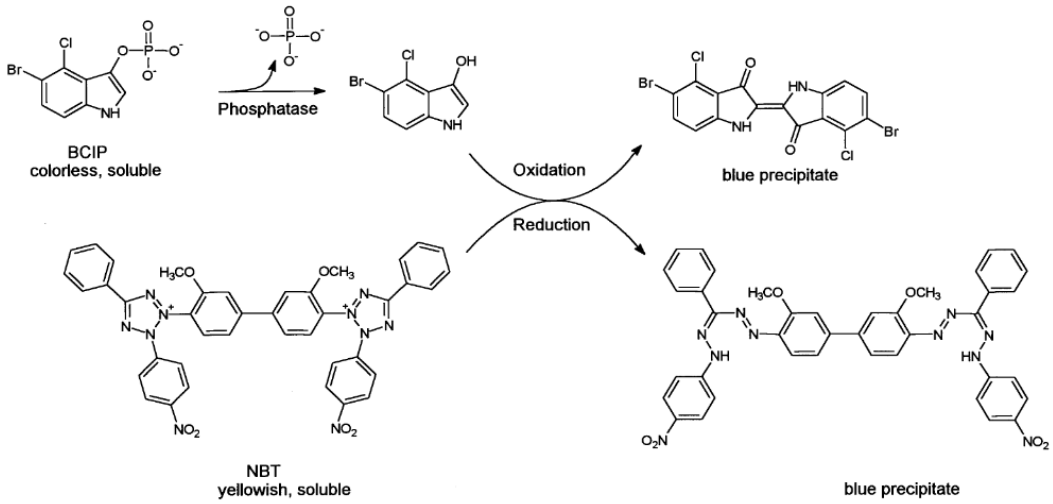

Fig. 1: Mechanism for the dye-generating redox reaction.

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.
Update to include new safety Information to ensure handling according controlled conditions.

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-β-Gal Conjugate, 500 U, *Not available in US*	11 112 481 001
	Streptavidin-POD Conjugate, 500 U	11 089 153 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
BCIP	3 ml, 150 mg	11 383 221 001
	250 mg	10 760 994 001
	1 g	11 585 002 001

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.

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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