

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

Nitroblue tetrazolium chloride (NBT)

Solution

Cat. No. 11 383 213 001

3 ml

 Version 19

Content version: March 2019

Store at -15 to -25°C

Product overview

Commercial availability

Solution (100 mg/ml) in 70% dimethylformamide (DMF) (v/v).

The color of the NBT solution can vary between yellow and brown depending on the lot used.

Note: The color does not impair the quality or the function of the reagent. In some cases a precipitate may occur which is easily brought into solution by briefly warming the substrate at +37°C.

Properties

Formula	C ₄₀ H ₃₀ Cl ₂ N ₁₀ O ₆
Molecular weight	817.7

Spectral properties

NBT	$\lambda_{\text{max}}(\text{H}_2\text{O})$	= 257 nm	$\epsilon = 72600$ [l × mol ⁻¹ × cm ⁻¹]
Diformazane	$\lambda_{\text{max}}(\text{nitrobenzine})$	= 605 nm	$\epsilon = 40200$ [l × mol ⁻¹ × cm ⁻¹]

Description

NBT is a potent redox indicator forming an insoluble diformazane upon reduction ($E^{\circ} = +50$ mV) (1). The preparation is especially used if the dye developed should be stable for a prolonged time or if as 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, e. g. 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 autoxidizable. The reduction of tetrazoliumchloride in competition with O₂ is favoured in the presence of a detergent (e.g. Triton[®] X-100* or Tween[®] 80*) in a 3-fold CMC-concentration (1).

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 e.g. after blotting procedures on appropriate supports (e.g. nylon membranes, nitro-cellulose etc.) for the detection of nucleic acids, proteins or glycoconjugates.

Application

NBT is used as redox indicator in combination with BCIP for the sensitive detection of alkaline phosphatase.

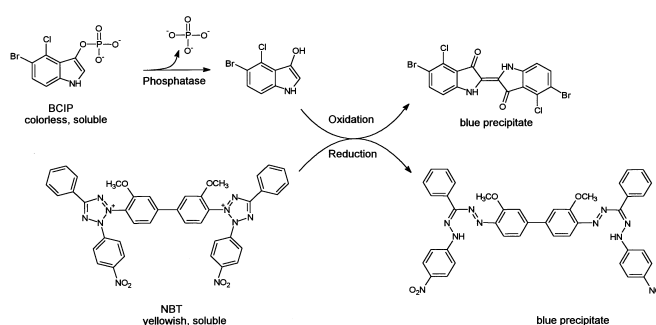
Both dyes have very little solubility in water or lipid and can be applied for the AP-detection in

- immunoblotting (4) and
- immunohistochemical (5) assays.

Reaction scheme

BCIP (3) is the AP-substrate which reacts further after the dephosphorylation to give a dark-blue indigo-dye as an oxidation product. NBT (1) serves herein as the oxidant and gives also a dark-blue dye. It intensifies thereby the color and makes the detection more sensitive.

Scheme for the dye-generating redox reaction:



Storage/ stability

Stable at -15 to -25°C until the expiration date printed on the label protected from light.

Preparation of staining solution

Always prepare this solution fresh shortly before use!

- For all applications except DIG System: Add 50 μ l NBT solution and 375 μ l BCIP to 10 ml 0.1 M Tris-HCl, pH 9.5 (+20°C), 0.1 M NaCl, 0.05 M MgCl₂.
- For DIG System applications: Add 50 μ l NBT solution and 375 μ l BCIP to 10 ml 0.1 M Tris-HCl, pH 9.5 (+20°C), 0.1 M NaCl.

Note: Do not include MgCl₂ in the detection buffer as this might lead to spotty background on the membrane after the detection procedure.

Immunodetection of digoxigenin-labeled biomolecules

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

Please follow the pack inserts (available on request and on our web site) 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*,
- DIG Glycan Detection Kit*,
- DIG Protein Detection Kit*,
- DIG Glycan Differentiation Kit*.

The staining solution mentioned above can substitute the individual staining solutions in these kits.

Changes to Previous Version

Editorial changes.

Immunodetection of biotin-labeled glycoconjugates and proteins

Additional required reagents

- TBS: 0.05 M Tris-HCl*, 0.15 M NaCl, pH 7.5.
- Streptavidin-AP-conjugate*
- Tween 20*
- BCIP*
- Blocking solution: Dissolve 0.5 g Blocking reagent* in 100 ml TBS, pH 7.5, by heating to +50 to +60°C (1 h). The dissolution can be accelerated by sonication or by incubation in a microwave oven. The solution remains turbid.

Protocol

Incubate all filters by gentle agitation at +15 to +25°C except for color development which should be done without any motion.

Note: The volumes state below refer to a 50 - 100 cm² filter.

Step	Action
1	Incubate the filter with the immobilized biotin-labeled samples for at least 30 min on approx. 20 ml Blocking solution. (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.)
2	Wash 3 times for 10 min each with approx. 50 ml TBS.
3	Incubation with streptavidin-AP: Add 5 µl of the conjugate to 10 ml TBS, 0.1% Tween 20 (w/v) and incubate the filter with this solution for 1 h.
4	Wash 3 times for 10 min each with approx. 50 ml TBS.
5	Staining reaction: Staining solution (mix just before use): 10 ml 0.1 M Tris buffer, pH 9.5, 0.05 M MgCl ₂ , 0.1 M NaCl, 50 µl NBT solution, 37.5 µl BCIP solution.
6	Immerse the filter without agitation in the staining solution and observe the development of the blue color. The development is normally completed 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 double dist. water to stop staining then dry the filter on paper towels. The filter can now be photographed or photocopied directly and can be stored under light protection for documentation.

In Situ Hybridisation

For non-radioactive *in situ* Hybridization with alkaline phosphatase and NBT/BCIP chromogen it is important **not to** use xylene-based mounting media because these could lead to crystal formations of the color precipitates.

References

- 1 Michal, G. et al. in: Methods of Enzymatic Analysis (Bergmeyer, U. ed.), 3rd ed. 1983, Vol. 1., p. 197-232.
- 2 Altman, F.P. (1976) Tetrazolium Salts and Formazans, "Absorption characteristics, p. 28-29, Gustav Fischer Verlag Stuttgart, New York
- 3 Horwitz, J. P. et al. (1966), *J. Med. Chem.* **9**, 447.
- 4 Wolf, P. L. et al (1968), *Enzymologia (Enzymas)* **35**, 154.
- 5 Wolf, P. L. et al (1973), *Clin. Chem.* **19**, 1248.

Available Printed Materials

DIG Appl. Manual for FilterHybridization
Nonradioactive *In Situ* Hybridization Manual
DIG Product Selection Guide

Note:

The product is toxic, it contains dimethylformamide: R61-20/21-36 S 53-45

May cause harms to the unborn child. Avoid exposure - obtain special instructions before use.

Ordering Information

Kits

Product	Pack Size	Cat. No.
DIG High Prime DNA Labeling and Detection Starter Kit I	1 kit (12 labeling reactions and 24 detection reactions)	11 745 832 910
DIG DNA Labeling and Detection Kit	1 kit (25 labeling reactions and 50 blots)	11 093 657 910
DIG Nucleic Acid Detection Kit	1 kit (40 blots)	11 175 041 910
DIG Glycan Detection Kit	1 kit	11 142 372 001
DIG Glycan Differentiation Kit	1 kit	11 210 238 001
DIG RNA Labeling Kit	1 kit	11 175 025 910
DIG RNA Labeling Mix	40 µl (20 reactions)	11 277 073 910

Single reagents

Product	Pack Size	Cat. No.
ABTS	2 g	10 102 946 001
ABTS 50 mg/ tablet	20 tablets	11 112 422 001
ABTS, ready-to-use solution	3 x 100 ml	11 684 302 001
Anti-DIG-AP, Fab fragments	150 U (200 µl)	11 093 274 001
Anti-FLUOS-AP, Fab fragments	150 U	11 426 338 001
BCIP, 4-toluidine salt, solution	3 ml (150 mg)	11 383 221 001
BCIP, 4-toluidine salt, powder	250 mg 1 g	10 760 994 001 11 585 002 001
BM Blue POD Substrate, soluble ready-to-use solution	100 ml	11 484 281 001
BM Purple AP Substrate precipitating ready-to-use	100 ml	11 442 074 001
CDP-Star	1 ml 2 x 1 ml	11 685 627 001 11 759 051 001
CDP-Star, ready-to-use	2 x 50 ml	12 041 677 001
CSPD	1 ml 2 x 1 ml 4 x 1 ml	11 655 884 001 11 759 035 001 11 759 043 001
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
Fast Red Tablets	20 tablets	11 496 549 001
INT/BCIP Stock Solution	3 ml	11 681 460 001
NBT, crystals	1 g 5 g	11 087 479 001 11 585 029 001
NBT/BCIP ready-to-use Tablets	20 tablets	11 697 471 001
Streptavidin-AP	150 U	11 093 266 910
Tween 20	5x 10 ml	11 332 465 001

* available from Roche Diagnostics

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