

NBT/BCIP Stock Solution

Cat. No. 11 681 451 001

8 ml

Version 08

Content version: April 2016

Store at +2 to +8°C

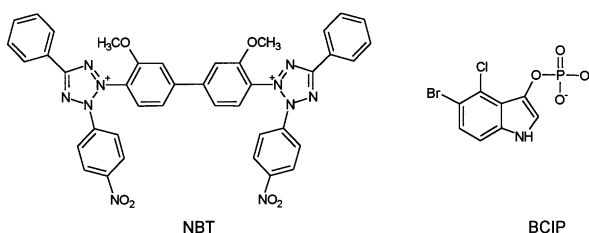
Product overview

- Content**
- Solution of
- 18.75 mg/ml NBT (Nitro blue tetrazolium chloride) and
 - 9.4 mg/ml BCIP (5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt) in 67% DMSO (v/v).
- Note:** The color of the solution can vary between yellowish to light brown.

Properties

	NBT	BCIP
Formulas	$C_{40}H_{30}Cl_2N_{10}O_6$	$C_8H_6NO_4 \text{ BrClIP} \times C_7H_9N$
Molecular weight	817.7	433.6

Structures



Application

The solution is used for the sensitive detection of alkaline phosphatase.

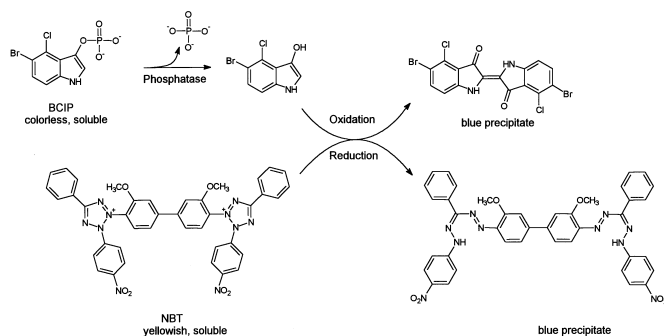
Both dyes have very little solubility in water or lipid and can be applied for the

- AP-detection in immunoblotting (3) and
- immunohistochemical (4) assays.

Reaction scheme

BCIP (1) is the AP-substrate which reacts further after the dephosphorylation to give a dark-blue indigo-dye as an oxidation product. NBT (2) 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

The unopened reagent is stable at +2 to +8°C until the expiration date printed on the label or at least for 4 weeks at +15 to +25°C stored protected from light.

Note: If precipitates occur in the color substrate solution, they can be removed by warming up the solution to +50°C. If the precipitates do not resolve centrifuge the tube prior to use because the precipitates might cause background; centrifugation will not reduce the overall sensitivity.

Preparation of staining solution

Always prepare this solution fresh shortly before use!

- For all applications except DIG System: Add 200 µl of the stock solution 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 200 µl of the stock solution 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 Instructions for Use (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 Differentiation Kit.

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

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*
- 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 +4°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	<ul style="list-style-type: none">Immerse the filter without agitation in the staining solution (preparation see above) 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 Horwitz, J. P. *et al.* (1966), *J. Med. Chem.* **9**, 447.
- 2 Michal, G. *et al.* in *Methods of Enzymatic Analysis* (Bergmeyer, H. U. ed.) 3rd edition 1983, Vol 1, 197.
- 3 Wolf, P. L. *et al.* (1968), *Enzymologia (Enzyas)* **35**, 154.
- 4 Wolf, P. L. *et al.* (1973), *Clin. Chem.* **19**, 1248.
- 5 Nonradioactive In Situ Hybridization Application Manual - 3rd edition (2002)Roche Applied Science

Changes to previous version

Editorial changes

Ordering Information

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 Differentiation Kit	1 kit	11 210 238 001
DIG RNA Labeling Kit	1 kit (2 x 10 labeling reactions)	11 175 025 910
DIG RNA Labeling Mix	40 µl (20 reactions)	11 277 073 910

Single reagents

Product	Pack Size	Cat. No
NBT/BCIP Ready-to-Use Tablets	20 tablets	11 697 471 001
NBT, solution	3 ml (300 mg)	11 383 213 001
NBT, crystals	5 g	11 585 029 001
BCIP, disodium salt, crystals	1 g	11 017 373 001
BCIP, 4-toluidine salt, powder	250 mg 1 g	10 760 994 001 11 585 002 001
BCIP, 4-toluidine salt, solution	3 ml (150 mg)	11 383 221 001
INT/BCIP Stock Solution	3 ml	11 681 460 001
BM Purple AP Substrate, precipitating	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
Anti-Digoxigenin-AP, Fab fragments, from sheep	150 U (200 µl)	11 093 274 001
Streptavidin-AP	150 U 1000 U (1 ml)	11 093 266 001 11 089 161 001
Anti-Fluorescein-AP, Fab fragments	150 U	11 426 338 001

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