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

BCIP
4-toluidine salt

Version: 10
Content Version: November 2020

Cat. No. 11 383 221 001 3 ml
150 mg

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	BCIP	Solution (50 mg/ml) in dimethylformamide (DMF).	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	BCIP	Store at −15 to −25°C. ⚠ ***Keep protected from light.***

1.3. Additional Equipment and Reagent required

For immunodetection of digoxigenin-labeled biomolecules

- DIG Nucleic Acid Detection Kit*

For immunodetection of biotin-labeled glycoconjugates and proteins

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*
- Nitro blue tetrazolium chloride (NBT), solution*
- Blocking Reagent*
- Maleic acid buffer
- DIG Wash and Block Buffer Set* (optional)
- Tween 20*
- Double-distilled water
- Heating block or microwave oven

1.4. Application

Use BCIP for alkaline phosphatase detection in:

- PAGE
- Immunoblotting
- Immunohistochemistry
- Tissue sections

2. How to Use this Product

2.1. Before you Begin

Safety Information

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

Solution	Preparation/Composition	Storage and Stability
Blocking solution for nonfilter hybridization	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.*	–
Blocking stock solution, 10x for filter hybridization	Dissolve Blocking Reagent in Maleic acid buffer (100 mM Maleic acid, 150 mM NaCl, pH 7.5 [+20°C], adjusted with concentrated or solid NaOH, sterile) to a final concentration of 10% (w/v) with shaking and heating either on a heating block or in a microwave oven.	Autoclave stock solution and store at +2 to +8°C or −15 to −25°C.
1x Blocking solution	Dilute the 10x Blocking stock solution with 1x Maleic acid buffer to a 1x-concentrated solution.	⚠ ***Always prepare fresh.***
Staining solution	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.	

2.2. Protocols

Immunodetection of digoxigenin-labeled biomolecules

The procedure for the immunological detection of digoxigenin-labeled biomolecules is described in the Instructions for Use of the DIG Nucleic Acid Detection Kit*.

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 1x Blocking solution.
   ⓘ *If necessary, the detection can be interrupted at this stage and the filter kept in the 1x 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 gray to almost black 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.

2.3. Parameters

Chemical Formula

$C_8H_6NO_4BrClP \times C_7H_9N$

Chemical Name

Chemical structure

BCIP (5-Bromo-4-chloro-3-indolyl-phosphate)

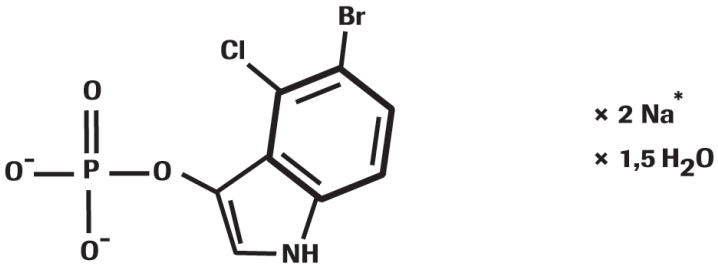

Fig. 1: Chemical structure of BCIP.

Molecular Weight

BCIP toluidine salt: 433.6 g/mol

3. Additional Information on this Product

3.1. Test Principle

How this product works

BCIP serves as substrate for alkaline phosphatase. The 5-bromo-4-chloro-3-indoxyl formed reacts spontaneously with O_2 to give an insoluble purple indigo dye. Instead of oxygen, other electron acceptors can be used, such as nitro blue tetrazolium chloride (NBT) (Figure 2).
If both substrates are used in combination, an enhanced color development is observed. Both dyes formed are insoluble in aqueous systems and lipids, so that the method is applicable for the AP detection in PAGE as well as for immunoblotting and immunohistochemistry. The indolyl substrate offers precise enzyme localization with very little or no diffusion of the insoluble products in tissue sections.

Reaction principle

BCIP is used as the substrate for alkaline phosphatase in combination with nitro blue tetrazolium chloride (NBT) as the electron acceptor. Substrates and reaction products of alkaline phosphatase catalyze the color reaction with NBT/BCIP, see Figure 2.

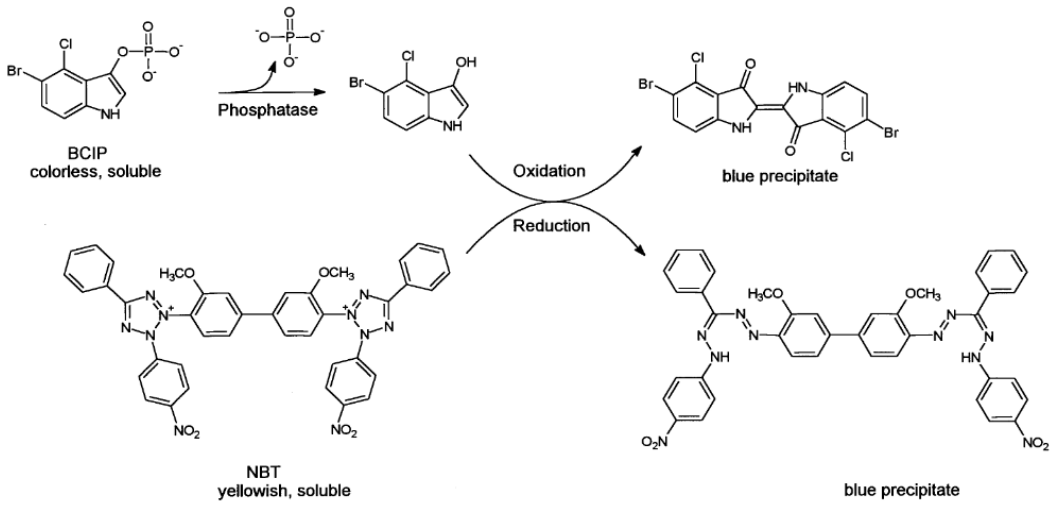

Fig. 2: Mechanism for the dye-generating redox reaction.

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
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
4-Nitro blue tetrazolium chloride (NBT)	3 ml, 300 mg	11 383 213 001
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001

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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