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

INT/BCIP Stock Solution

Version: 08
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Cat. No. 11 681 460 001 3 ml

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	INT/BCIP Stock Solution	Solution with 33 mg/ml INT (2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride) and 33 mg/ml BCIP (5-bromo-4-chloro-3-indolyl phosphate, toluidine salt in DMSO).	1 bottle, 3 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	INT/BCIP Stock Solution	Store at +2 to +8°C. ⚠ ***Keep protected from light.***

1.3. Additional Equipment and Reagent required

For the preparation of the staining solution

See section, **Working Solution** *for additional information on preparing solutions.*

- Tris-buffer
- $MgCl_2$
- NaCl

For immunodetection of DIG-labeled biomolecules

See section, **Working Solution** *for additional information on preparing solutions.*

- TBS: 0.05 M Tris-HCl*, 0.15 M NaCl, pH 7.5
- Strepatvidin-AP-conjugate*
- Tween 20*
- Blocking Reagent*
- Double-distilled water

1.4. Application

Use INT/BCIP for alkaline phosphatase detection in:

- Southern blots
- Western blots
- Immunohistochemistry
- Immunocytochemistry

2. How to Use this Product

2.1. Before you Begin

General Considerations

Both dyes show low solubility in water or lipids. Because the overall color is a reddish brown, the staining solution can be used as an alternative to NBT/BCIP (blue color) for the AP-detection in immunoblotting and immunohistological assays, although it is less sensitive than NBT/BCIP.

- Because of the color, different antigens can be detected simultaneously, such as in immunoblotting assays.
- For the detection of the second antigen, the development of a blue color should be used so that one can localize both antigens simultaneously at the very end on the same membrane.
- When both color developing reactions occur at the same location, the blue color will be dominant, enabling, for example, the visualization of a distinct antigen or glycoprotein and total protein (using INT/BCIP). For best results, first detect with NBT/BCIP, then with INT/BCIP, and then Fast Red.
- Color precipitates can be removed by washing with methanol.

Working Solution

Solution	Preparation/Composition	Storage and Stability
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.*	Store at +15 to +25°C.
TBS	Prepare a 0.05 M Tris-HCl*, 0.15 M NaCl, pH 7.5 buffer.	Store at +2 to +8°C.
Antibody solution	Add 5 ml of the Streptavidin-AP* conjugate to 10 ml TBS, Tween 20*, 0.1% (w/v).	–
Staining solution (10 ml)	▪ Equilibrate the INT/BCIP Stock Solution to +15 to +25°C until liquefied. ▪ 10 ml 0.1 M Tris buffer*, pH 9.5, 0.05 M $MgCl_2$, 0.1 M NaCl, 75 µl INT/BCIP Stock Solution.	⚠ ***Always prepare fresh.***

2.2. Protocols

Immunodetection of biotin-labeled biomolecules

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

⚠ ***Incubate the filter 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. Incubate the filter in the Streptavidin-AP conjugate 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 under light protection for documentation.

Prevent the membranes from getting dry if you intend to strip and reprobe.

2.3. Parameters

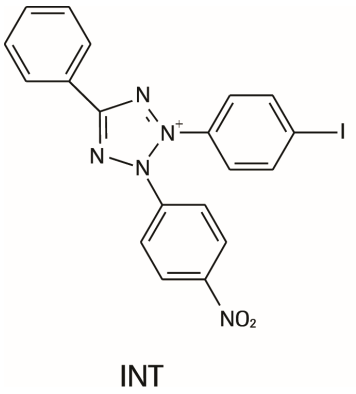
Chemical Formula

INT: $C_{19}H_{13}ClIN_5O_2$
BCIP: $C_8H_6NO_4BrClP \times C_7H_9N$

Chemical Name

Chemical structure INT

INT: 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride

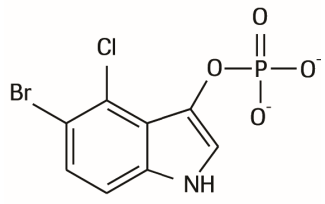


INT

Fig. 1: Chemical structure of INT.

Chemical structure BCIP

BCIP: 5-bromo-4-chloro-3-indolyl phosphate



BCIP

Fig. 2: Chemical structure of BCIP.

Molecular Weight

INT: 505.7 g/mol
BCIP: toluidine salt: 433.6 g/mol

3. Additional Information on this Product

3.1. Test Principle

How this product works

1. 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 2-[4-Iodophenyl]-3-[4-nitrophenyl]-5-phenyl-tetrazolium chloride (INT).

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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 alkaline phosphatase detection immunohistochemistry.

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3. 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 INT as the electron acceptor. Substrates and reaction products of alkaline phosphatase catalyze the color reaction with INT/BCIP, see Figure 3.

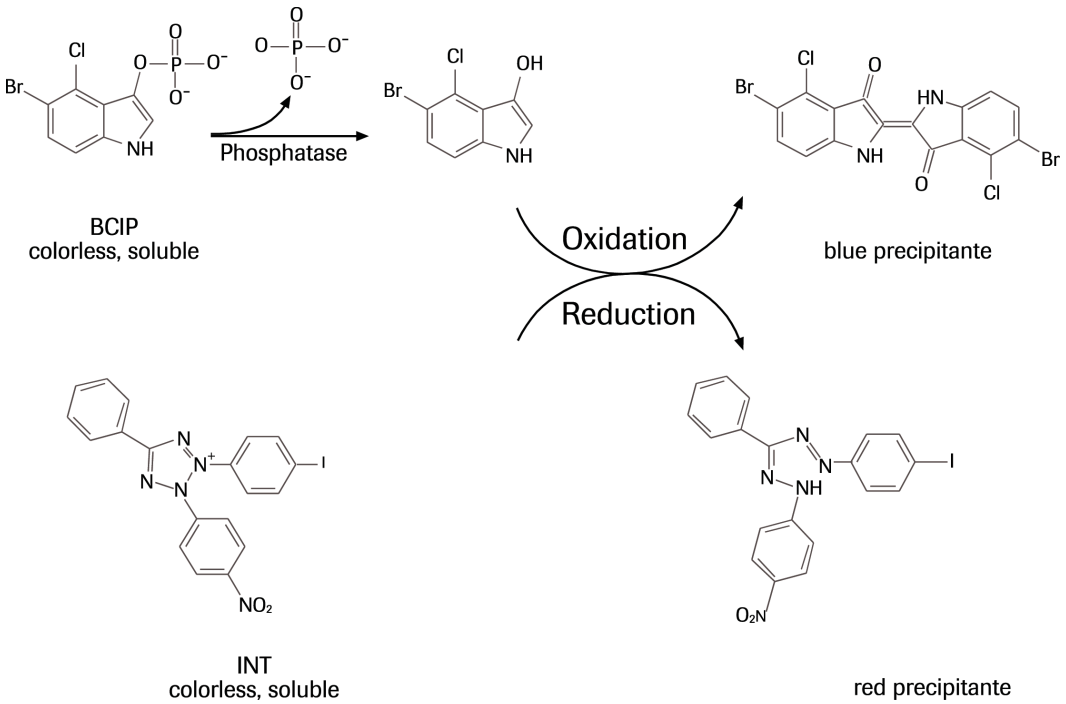

Fig. 3: Reaction mechanism of the dye-generating redox reaction.

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.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris hydrochloride	500 g	10 812 846 001
Streptavidin Conjugates	Streptavidin-AP Conjugate, 1,000 U	11 089 161 001
	Streptavidin-POD Conjugate, 500 U	11 089 153 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Blocking Reagent	50 g	11 096 176 001

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 and select the corresponding product catalog.

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

1121.11682296001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany