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## Product Information

### Nitrotetrazolium blue chloride

Product Numbers **N6876** and **N6639**

Storage Temperature 2–8 °C

CAS<sup>#</sup> 298-83-9

Synonyms: 2,2'-bis(4-Nitrophenyl)-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride; 3,3'-(3,3'-Dimethoxy-4,4'-biphenylene)bis[2-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride]; *p*-Nitro-Blue tetrazolium chloride; *p*-Nitrotetrazolium blue; NBT; Nitro BT

### Product Description

Molecular Formula:  $C_{40}H_{30}N_{10}O_6 \cdot 2Cl$

Formula Weight: 817.64

Melting Point:

205 °C<sup>1</sup>, 189–192 °C<sup>2</sup> (decomposes)

Extinction Coefficients:

260 nm:  $E^{1\%}_1 = 740$  (in water)<sup>1</sup>

257 nm: Molar Extinction Coefficient = 61,300 (in water)<sup>2</sup>

NBT is prepared synthetically.<sup>3,4</sup> The most common application for NBT is the detection of alkaline phosphatase on western blots.<sup>5</sup> NBT has also been used as a redox indicator for other enzymatic reactions including dehydrogenases,<sup>6</sup> threonine deaminase,<sup>7</sup> glucose-6-phosphate dehydrogenase,<sup>8</sup> phosphofructokinase on polyacrylamide gels,<sup>9</sup> oxidases on polyacrylamide gels,<sup>10</sup> and pentose shunt dehydrogenases.<sup>11</sup> Redox and halfwave potentials have been determined for NBT.<sup>12,13</sup> NBT has also been used as a colorimetric indicator of bacterial infection in blood samples.<sup>15</sup>

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

NBT is soluble in H<sub>2</sub>O at 10 mg/ml, ethanol at 5 mg/ml and 2-methoxyethanol at 20 mg/ml.<sup>1</sup> A stock solution at 10 mg/ml in water is stable 1–2 weeks in the dark at 2–8 °C.

### Storage/Stability

NBT has a shelf life of three years when stored at 2–8 °C and protected from light.

### Procedure

#### The NBT/BCIP System for Detection of Alkaline Phosphatase

Nitro Blue Tetrazolium (NBT) is used with the alkaline phosphatase substrate 5-Bromo-4-Chloro-3-Indolyl Phosphate (BCIP) in immunoblotting<sup>5</sup> and immunohistological<sup>14</sup> staining procedures. This substrate system produces an insoluble NBT diformazan end product that is blue in color and can be observed visually.

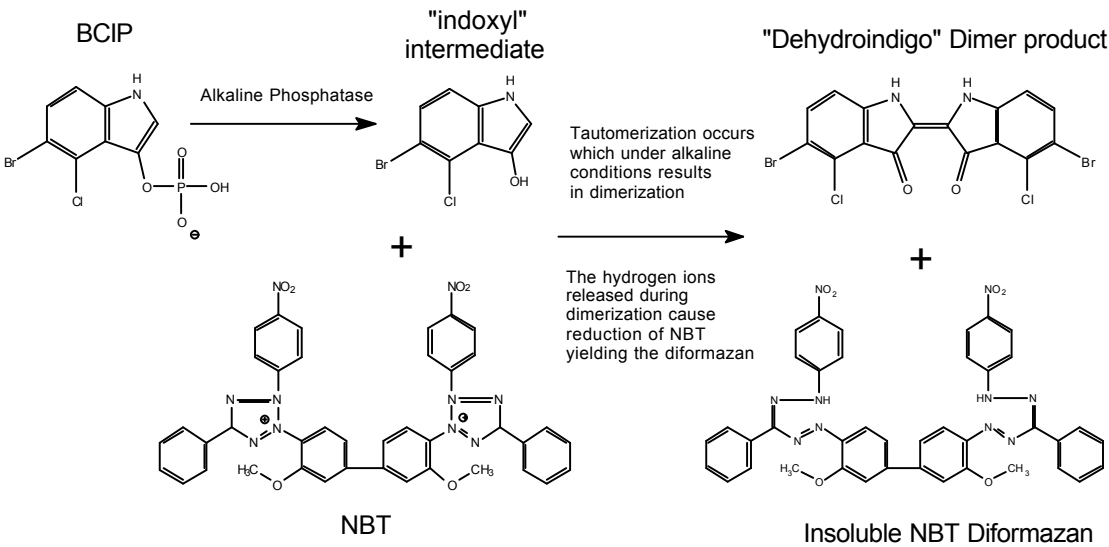
The standard protocol for western blotting is as follows:

1. Prepare substrate buffer: 0.1 M Tris, 100 mM sodium chloride, 5 mM MgCl<sub>2</sub>, pH 9.5, adjust pH with HCl.
2. Prepare NBT stock solution at 10 mg/ml in water.
3. Prepare BCIP, Product No. B6149, stock solution at 50 mg/ml in water.
4. Add 33 µl of a 50 mg/ml stock solution of BCIP in water and 330 µl of a 10 mg/ml NBT stock solution in water to 10 ml of substrate buffer.
5. Rinse specimens incubated with an alkaline phosphatase conjugate in a wash buffer (non-phosphate) before treatment with the BCIP/NBT substrate solution. Cover the entire specimen with the reagent during color development.
6. Incubate the specimen at room temperature with the BCIP/NBT reagent for approximately 10 minutes. Specimens and procedure may affect the length of time needed for color development.
7. Monitor color development to avoid over-development. Stop color development by rinsing the specimen with distilled water.

**Troubleshooting for Western Blotting:**

Problem	Suggestion
The background is too high.	Use a blocking step prior to the application of the primary antibody. Normal serum (10% v/v) from the same species as the second antibody generally produces the best results.
	Additional blocking agents for immunoblotting are 10% BSA, 0.05% Tween <sup>®</sup> 20, or 3% non-fat dried milk. Note: Do not use milk as a blocking agent when using avidin-biotin systems.
	Decrease staining time.
	Titer the conjugate to optimize working dilution.
No color develops or color is too faint.	Adjust the concentration of the primary antibody.
	Adjust the concentration of the secondary antibody.
	Determine if the enzyme conjugate is active.
	Consider using an amplifying system such as avidin-biotin.
	Increase the staining time.
	Adjust the transfer time of the samples to the nitrocellulose membrane.
	Increase the amount of sample.

**BCIP/NBT Reactions**



## References

1. Green, F.J. (ed.) The Sigma-Aldrich Handbook of Stains, Dyes & Indicators (Aldrich Chemical Co., Milwaukee, WI), p. 523 (1990).
2. Altman, F.P., Tetrazolium salts: a consumer's guide. *Histochemical J.*, **8**, 471 (1976).
3. Kwan-Chung, T., *et al.*, Syntheses of some p-nitrophenyl substituted tetrazolium salts as electron acceptors for the demonstration of dehydrogenases. *JACS*, **78**, 6139-6144 (1956).
4. Karmarkar, S.S., *et al.*, Synthesis of p-nitrophenyl substituted tetrazolium salts containing iodine and other groups. *JACS*, **81**, 3771 (1959).
5. Blake, M.S., A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. *Anal. Biochem.*, **136**, 175 (1984).
6. Fine, I.H. and Costello, L.A., The use of starch electrophoresis in dehydrogenase studies. *Meth. Enzymol.*, **6**, 958 (1963).
7. Feldberg, R.S. and Datta, P., Threonine deaminase: a novel activity stain on polyacrylamide gels. *Science*, **170**, 1414-1415 (1970).
8. Criss, W.E. and McKerns, K.W., Purification and partial characterization of glucose 6-phosphate dehydrogenase from cow adrenal cortex. *Biochemistry*, **7**, 125-134 (1968).
9. Brock, D.J.H., Purification and properties of sheep liver phosphofructokinase. *Biochem. J.*, **113**, 235-242 (1969).
10. Feinstein, R.N. and Lindahl, R., Detection of oxidases on polyacrylamide gels. *Anal. Biochem.*, **56**, 353-360 (1973).
11. Altmann, F.P., The use of eight different tetrazolium salts for a quantitative study of pentose shunt dehydrogenation. *Histochemie*, **19**, 363-374 (1969).
12. Horwitz, J.P., *et al.*, Substrates for Cytochemical demonstration of enzyme activity. II. Some Dihalo-3-indolyl phosphates and sulfates. *J. Med. Chem.*, **9**, 447 (1966).
13. Karmarkar, S.S., *et al.*, Preparation of nitrotetrazolium salts containing benzothiazole. *J. Org. Chem.*, **25**, 575-585 (1960).
14. Bergmeyer, H. U., *Methods of Enzymatic Analysis*, 3rd ed., **1**, 198 (1983).
15. Park, B.H., *et al.*, Infection and nitroblue-tetrazolium reduction by neutrophils. A diagnostic acid. *The Lancet* **2**, 532-4 (1968).

## Related Products

### BCIP/NBT Combination

- SIGMAFAST™ BCIP/NBT tablet (each tablet prepares 10 ml), Product No. B5655
- BCIP/NBT Liquid Substrate System, Product No. B1911
- BCIP/NBT-Purple Liquid Substrate System for Membranes, Product No. B3679
- BCIP/NBT-Blue Liquid Substrate System for Membranes, Product No. B3804
- BCIP/NBT solution, premixed, Product No. B6404
- ProteoQwest™ Colorimetric Western Blotting Kit, BCIP/NBT Substrate for Mouse Monoclonal IgG Antibodies, Product No. PQ0111

### NBT

- Nitro Blue Tetrazolium tablet (10 mg substrate per tablet), Product No. N5514

### BCIP

- 5-Bromo-4-chloro-3-indolyl phosphate disodium, Product No. B6149
- 5-Bromo-4-chloro-3-indolyl phosphate disodium salt for molecular biology, Product No. B1026
- 5-Bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt, Product No. B8503
- 5-Bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt for molecular biology, Product No. B6777
- 5-Bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt, tablet (25 mg substrate per tablet), Product No. B0274

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