

BCIP

34040

0155.2

Number**Description**

34040

BCIP (5-bromo-4-chloro-3'-indolylphosphate *p*-toluidine salt), 1g

Storage: Upon receipt store product at -20°C protected from moisture. Product is shipped at ambient temperature.

Introduction

An ideal system for blotting or staining applications is the combination of Thermo Scientific BCIP (5-bromo-4-chloro-3'-indolylphosphate *p*-toluidine salt) and NBT (nitro-blue tetrazolium chloride). Together they yield an intense, insoluble black-purple precipitate when reacted with alkaline phosphatase. This reaction proceeds at a steady rate, allowing accurate control of sensitivity and development of the reaction.

Protocol for Staining with BCIP and NBT**A. Materials Required**

- Dimethylformamide (DMF, Product No. 20673).
- NBT (nitro-blue tetrazolium chloride, Product No. 34035) Stock Solution: Dissolve 0.5g of NBT in 10mL of 70% DMF in water. Stock is stable at 4°C for ~1 year.
- BCIP Stock Solution: Dissolve 0.5g of BCIP in 10mL 100% DMF. Stock is stable at 4°C for ~1 year.
- Substrate Buffer: 100mM diethanolamine buffer (pH 9.5) containing 100mM NaCl and 5mM MgCl₂, or use Diethanolamine Substrate (5X) Buffer (Product No. 34064).

B. Method

1. To prepare the NBT/BCIP Solution, add 66µL of stock NBT to 10mL of Substrate Buffer and mix well. Add 33µL of stock BCIP to the solution. Use the NBT/BCIP Solution within 1 hour.
2. Place blot in a suitable container and add the NBT/BCIP Solution. Use 10mL of solution for a 15 × 15cm² membrane. Incubate blot at room temperature with agitation until the bands are suitably dark. Typically, ~30 minutes is required for development.
3. Remove the NBT/BCIP Solution and rinse blot with water to stop the reaction.

Related Thermo Scientific Products

34035	NBT (nitro-blue tetrazolium chloride), 1g
34042	1-Step™ NBT/BCIP, 250mL
31872	Normal Goat Serum, 2mL
31884	Normal Rabbit Serum, 2mL
32040	ABC Standard Alkaline Phosphatase Staining Kit
31002	NeutrAvidin® Protein, Alkaline Phosphatase Conjugated, 2mg
21323	Streptavidin, Alkaline Phosphatase Conjugated, 3mg

General References

- Altman, F.P. (1976). Tetrazolium salts and formazans. *Prog Histochem Cytochem* **9**:1-56.
- Cordell, J.L., *et.al.* (1984). Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP Complexes). *Histochem Cytochem* **32**:219-29.
- Leary, J.J., *et al.* (1983). Rapid and sensitive, colorimetric method or visualizing biotin-labeled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose: bio-blots. *Proc Natl Acad Sci USA* **80**:4045-9.

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