

Product Information

BCIP®/NBT Solution, premixed**B6404****Product Description**

5-bromo-4-chloro-3-indolyl phosphate (BCIP®) and nitro blue tetrazolium (NBT) are reagents which are widely used in tandem to detect alkaline phosphatase conjugates.¹⁻³ This premixed BCIP®/NBT solution is a substrate solution that is designed to visualize alkaline phosphatase conjugates in Western blotting. Several dissertations⁴⁻¹³ have cited use of product B6404 in their protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagent

This premixed BCIP®/NBT solution contains 0.48 mM NBT, 0.56 mM BCIP®, 10 mM Tris HCl (pH ~9.2), and 59.3 mM MgCl₂.

Additional Reagents Required

(Not provided)

- 10× Tris Buffered Saline (Cat. No. T5912)
- TWEEN® 20 (Cat. No. P2287)
- BSA Fraction V powder (Cat. No. A9647)
- Nonfat-dried milk (Cat. No. M7409)

Storage/Stability

This product may be stored at room temperature.

Procedure

1. After the gel is transferred onto a blotting membrane, wash the membrane for 5 minutes with the Washing Solution (see Table 1).
2. Incubate the rinsed membrane with primary antibody diluted in Blocking Solution (see Table 1) for 2 hours at room temperature with gentle agitation. Use a dilution based on the manufacturer's recommendation. A blocking step prior to Step 2 is usually not necessary.

3. Wash the membrane for 5 minutes with the Washing Solution.
4. Incubate the washed membrane with a secondary antibody-alkaline phosphatase conjugate in blocking solution for 2 hours at room temperature with gentle agitation. (A 1:1000 dilution of antibody in blocking solution is recommended.)
5. Wash the membrane 3 times for 5 minutes each in Washing Solution.
6. Cover the membrane with the premixed BCIP®/NBT solution for 1-5 minutes at room temperature until the desired color is obtained. Use ~20 mL for a 10 × 10 cm membrane.
7. The color development can be stopped either:
 - (a) by extensive washing with water, or
 - (b) by rinsing with a 1% acetic acid solution.

Table 1. Commonly used washing and blocking solutions

Condition	Washing Solution	Blocking Solution
1	1× TBS (20 mM Tris-HCl, pH 7.5, and 0.9% NaCl)	Washing solution + 0.5% TWEEN® 20
2	1× TBS + 0.05% TWEEN® 20	Washing solution + 1-5% BSA
3	1× TBS + 0.05% TWEEN® 20	Washing solution + 2-4% nonfat-dried milk

Selection depends on the stringency required:

- Condition 1 is the least stringent.
- Condition 3 is most stringent.
- A version of Condition 3, using Washing Solution 3 and Blocking Solution 3 with 2% nonfat-dried milk, is recommended for most applications.

References

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