

Product Information

BCIP®/NBT solution, premixed

Catalog Number **B6404**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

The premixed BCIP®/NBT solution is a substrate solution designed for visualizing alkaline phosphatase conjugates in Western blotting.

Components

The premixed BCIP/NBT solution contains 0.48 mM NBT (nitro blue tetrazolium), 0.56 mM BCIP (5-bromo-4-chloro-3-indolyl phosphate), 10 mM Tris HCl, pH ~9.2, and 59.3 mM MgCl₂.

Reagents Required but Not Provided

10× Tris Buffered Saline (Catalog Number T5912)
TWEEN® 20 (Catalog Number P5927)
BSA Fraction V powder (Catalog Number A9647)
Nonfat-dried milk (Catalog Number M7409)

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.

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 by extensive washing with water or 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 and condition 3 is most stringent. Washing Solution 3 and Blocking Solution 3 with 2% nonfat-dried milk is recommended for most applications.

References

1. Scott, D.I., *J. of Immunological Methods*, **119** (1989) 153-187.
2. Batteiger, B., *J. of Immunological Methods*, **55** (1982) 297-307.
3. Miescher, F., *Analytical Biochemistry*, **119** (1982) 142-147.

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