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ProductInformation

4-CHLORO-1-NAPHTHOL SOLUTION

Product No. **C 8302**
Store at 2-8 °C

Product Description
Solution contains 0.48 mM 4-Chloro-1-Naphthol, 50 mM Tris-HCl and 0.2 M NaCl in 17% methanol. This is a substrate solution designed for visualizing horseradish peroxidase conjugates in Western Blotting.

Items not provided:
10X TBS (Product No. T 5912)
20% Tween 20 (Product No. P 9203)
BSA Fraction V powder (Product No. A 9647)
Nonfat dry milk (Product No. M 7409)

Instructions for Use:
1. After the gel is transferred onto a blotting membrane, wash the membrane for 5 minutes with the washing solution (See Table A for selection of appropriate solutions).
2. Incubate the rinsed membrane with primary antibody diluted in blocking solution (See Table A for selection of appropriate solutions) 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 secondary antibody peroxidase conjugate in blocking solution for 2 hours at room temperature with gentle agitation. (A 1:1000 dilution of antibody is recommended).
5. Wash the membrane 3 times for 5 minutes each in washing solution.
6. Add hydrogen peroxide (Product No. H 1009) to 4-Chloro-1-Naphthol Solution (Product No. C 8302) to obtain a final concentration of 0.01% hydrogen peroxide (v/v). Prepare immediately before use.
7. Cover the membrane with the substrate solution for 1-5 minutes at room temperature until the desired color is obtained. Use 10-20 ml for a 8x10 cm membrane. MAKE SURE THE SURFACE OF THE MEMBRANE IS COMPLETELY COVERED WITH SUBSTRATE SOLUTION.
8. The color development can be stopped by extensive washing with water. WATCH CAREFULLY DURING COLOR DEVELOPMENT TO AVOID OVER DEVELOPING.

TABLE A: Several commonly used washing and blocking solutions are listed. 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 milk is recommended for most applications.

Condition	Washing Solution	Blocking Solution
1	1X TBS*	Washing solution + 0.5% Tween 20
2	1X TBS + 0.05% Tween 20	Washing solution + 1-5% BSA
3	1X TBS + 0.05% Tween 20	Washing solution + 2-4% Nonfat dry milk

*1X TBS = 20 mM Tris-HCl. pH 7.5 and 0.9% NaCl

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

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