



Detection of Labeled Nucleic Acid Probes on Blots

PHOTOPROBE® Biotin- or FastTag®-labeled probes work well in membrane hybridization applications using colorimetric or chemiluminescence detection methods. In most experiments, probe concentrations of 10-100 ng/ml are routinely used. Hybridization solutions and conditions developed for radioactive probes can be used with PHOTOPROBE® Biotin- and FastTag®-labeled probes. For detailed information on pre-, post-, and hybridization steps, please refer to various molecular biology protocol manuals such as *"Molecular Cloning"*, J. Sambrook, E.F. Fritsch, T. Maniatis. 1989. Cold Spring Harbor Laboratory Press.

Protocol for Colorimetric Detection:

The following procedure is suggested as a guideline for the localization of labeled nucleic acids on nitrocellulose or nylon blots using alkaline phosphatase conjugates followed by a colorimetric protocol for visualization. (*Note: When using the VECTASTAIN® ABC Alkaline Phosphatase development system, the use of nitrocellulose is recommended.*)

- 1) Block the membrane in at least 1 ml/cm² 1x Vector® Casein Solution (Cat. No. SP-5020) for 30 minutes at room temperature with gentle shaking. The volume should be such that the blot is completely covered with blocking solution.
Note: Casein solution cannot be used when detecting Fucose-labeled nucleic acids with Alkaline Phosphatase-Aleuria aurantia lectin (Cat. No. MB-4100). Replace all casein solutions with TBST (100 mM Tris, pH 7.5, 0.15 M NaCl, 0.1% (v/v) Tween 20).
- 2) Dilute the alkaline phosphatase conjugate (antibody, lectin, streptavidin, or VECTASTAIN® ABC-AP reagent depending on which nucleic acid label is used) in 1x casein solution at the dilution recommended in the product specification sheet. Prepare at least 30 ml of diluted alkaline phosphatase conjugate per 100 cm² of membrane.
- 3) Incubate the membrane in the alkaline phosphatase-conjugate solution for 30 minutes at room temperature with gentle agitation.
- 4) Wash the membrane 3x 10 minutes in TBST.
Note: PBST (10 mM phosphate, pH 7.5, 0.15 M NaCl, 0.1% (v/v) Tween 20) can be substituted for TBST.

The alkaline phosphatase bound at the probe site on the membrane can then be visualized by formation of a colored precipitate as described below.

- 5) Transfer the blot to a different container for substrate development. Equilibrate the membrane for 5 minutes in 0.1 M Tris, pH 9.5.
Note: Always transfer membranes to a separate container for substrate development. If the same chamber is used for the enzyme conjugate and the substrate solution, background staining may occur.
- 6) Add 2 drops each of Vector® BCIP/NBT substrate kit (Cat. No. SK-5400) components 1, 2, and 3 to 5 - 10 ml of 0.1 M Tris, pH 9.5 (mix well after addition of each component). Scale up volume as necessary to give at least 0.1 ml/cm² of membrane.
- 7) Incubate the membrane in substrate until the desired level of detection is achieved.
- 8) Wash the membrane in deionized H₂O for 5 minutes to terminate the color development.
- 9) Air dry the blot and store protected from strong light.

Protocol for Chemiluminescence Detection:

1. Perform Southern or northern transfer and hybridization of biotinylated¹ probe using standard protocols. We recommend blotting onto nitrocellulose for optimal signal-to-noise ratio. Nylon membranes can be used but higher background may require shortening the exposure time, thereby reducing sensitivity.
2. Block blot in Vector® 1x Casein Solution (Cat. No. SP-5020) for 30 minutes at room temperature with gentle shaking. The volume should be such that the blot is completely covered with blocking solution.
3. Incubate the blot in Vector® 1x Casein Solution containing one of the following enzyme conjugates for detecting biotin-labeled probes:
 - 1.0 µg/ml AP-streptavidin (Cat. No. SA-5100)
 - 0.5 µg/ml AP-Anti-biotin (Cat. No. SP-3020)
 - 0.1 µg/ml HRP-streptavidin (Cat. No. SA-5004, or SA-5704)
 - 0.1 µg/ml HRP-Anti-biotin (Cat. No. SP-3010)

¹**Note:** Detection of other haptens (e.g. fluorescein, dinitrophenyl, digoxigenin, etc.) can be achieved using the appropriate AP- or HRP-conjugated antibody for that hapten. Please contact our Technical Service Department for assistance.

4. Wash blot 3 times for 15 minutes each in Wash Solution (100 mM Tris, pH 7.5, 150 mM NaCl, 0.05% SDS) at room temperature with gentle shaking.
5. Equilibrate blot for 5 minutes in Equilibration Buffer (100 mM Tris, pH 9.5, 150 mM NaCl).
6. Remove excess Equilibration Buffer by tipping blot on edge onto absorbent paper.
7. Place blot target-side-up on plastic wrap on a level surface.
8. For AP-based detection, pipet 0.1 ml/cm² of DuoLuX™ Chemiluminescent Substrate onto the blot surface. For HRP-based detection, just prior to use, mix DuoLuX™ Chemiluminescent Substrate with an equal volume of DuoLuX™ Peroxidase Converter Solution. Pipet 0.1 ml/cm² of this 1:1 mixture onto the blot surface. Incubate for 5 minutes under subdued light.
9. Briefly rinse the blot in Equilibration Buffer and remove standing liquid from the blot by pressing briefly between several sheets of filter paper or tissue (do not dry the blot completely).
10. Place the blot between pieces of acetate or plastic wrap and smooth away any bubbles trapped between the layers. Expose the blot to photographic film for the appropriate time. The long light emission characteristic of DuoLuX™ Chemiluminescent Substrate allows the user to re-expose the same blot, if necessary, until optimal signal to noise is achieved. Typical exposure times for nitrocellulose are approximately 3 to 30 minutes. For nylon, typical exposure times are 5 to 30 seconds. Blots can be re-exposed to photographic film up to 8 hours after incubation with DuoLuX™ Chemiluminescent Substrate.

Alternative Colorimetric Detection Systems:

Although alkaline phosphatase-based detection systems and BCIP/NBT substrate (Cat. No. SK-5400) provide the most sensitive color detection method, other enzyme-based systems can also be employed to detect PHOTOPROBE® Biotin or FastTag®-labeled probes. The following horseradish peroxidase-based reagents can be used to produce rapid and robust staining: Horseradish Peroxidase Streptavidin (Cat. No. SA-5004), VECTASTAIN® *Elite* ABC kit (Cat. No. PK-6100), Horseradish Peroxidase Avidin D (Cat. No. A-2004).

The most sensitive peroxidase substrates are tetramethylbenzidine (TMB, Cat. No. SK-4400), diamino-benzidine plus nickel chloride (DAB/Ni²⁺, Cat. No. SK-4100), Vector® VIP (SK-4600), and Vector® SG (SK-4700). A less sensitive substrate is 4-chloro-1-naphthol (4-CN, Cat. No. SK-4300). 4-CN is not recommended for use with nylon membranes.

Reference:

S. G. Daniel, M. E. Westling, M. S. Moss, B. D. Kanagy. 1998. FastTag® nucleic acid labeling system: A versatile method for incorporating haptens, fluorochromes, and affinity ligands into DNA, RNA, and oligonucleotides. *BioTechniques* 24: 484-489.