VECTOR LABORATORIES

# DuoLuX™

# Chemiluminescent/Fluorescent Substrate

## for Alkaline Phosphatase

Cat. No. SK-6605

### Introduction:

DuoLuX™ Chemiluminescent/Fluorescent Substrate is a novel acridan-based substrate that offers the convenience of using various chemiluminescent or fluorescent visualization methods. The DuoLuX™ Chemiluminescent/Fluorescent Substrate can be used in a number of protein and nucleic acid detection applications (Southern, northern, western or dot blotting, colony lifts, ELISA, etc.).

The DuoLuX™ Chemiluminescent/Fluorescent Substrate is available for either alkaline phosphatase (AP) or horseradish peroxidase (HRP) development. The choice of enzyme will depend on the application. Generally, AP will provide a higher signal to noise ratio than HRP and is, therefore, recommended for applications where optimal sensitivity is required. However, signal development is faster when using HRP. Thus, HRP may be preferred when digital imaging systems are used or when abundance of target reduces the need for a high signal to noise ratio.

DuoLuX™ Chemiluminescent/Fluorescent Substrate has very high sensitivity and prolonged light emission characteristics. This enables image documentation with either film or digital imaging systems. Unlike some chemiluminescent substrates, blots can be re-exposed to film as often as necessary over many hours. Because many digital imaging systems require a longer exposure time than film, the faster signal development of HRP relative to AP may be preferred when using these systems. Either PVDF, nitrocellulose or nylon membranes can be used, although the chemiluminescent signal develops faster on nylon and PVDF.

In addition to its chemiluminescent properties, the reaction product of the DuoLuX™ Substrate is also fluorescent. Fluorescence can be recorded with a digital imaging system or a conventional camera months after chemiluminescence has faded. For fluorescence detection, nitrocellulose is recommended. Acquisition of fluorescent signal requires a much shorter exposure time than chemiluminescence, often a fraction of a second.

For western blot or protein dot blot chemiluminescent applications, the sensitivity using AP is approximately 1 pg of target protein. Film exposure times are usually 1-5 minutes. For chemiluminescent western blots, DuoLuX™ Chemiluminescent/Fluorescent Substrate can be used on either nitrocellulose or PVDF membranes.

DuoLuX™ Chemiluminescent/Fluorescent Substrate can be used for nucleic acid detection in applications such as Southern and northern blots as well as plaque and colony screening. When using AP, approximately 100 fg of target nucleic acid can be detected. Typical film exposure times using AP are approximately 5 to 60 minutes. Signal can be developed on either nylon or nitrocellulose membranes. *See note E.*

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The DuoLuX™ Chemiluminescent/Fluorescent Substrate for alkaline phosphatase is supplied in a ready-to-use form consisting of 100 ml of reagent (Cat. No. SK-6605).

### DuoLuX™ chemiluminescence/fluorescence detection protocol for western blots:

For western and protein dot blot detection, the DuoLuX™ Substrate performs optimally using the reagents shown in the following protocol. These may be obtained individually or as part of the VECTASTAIN® ABC-AmP™ Chemiluminescence Detection Kits (Anti-Mouse IgG, Cat. No. AK-6602 or Anti-Rabbit IgG, Cat. No. AK-6601). Use of alternative reagents is possible, but may result in lower sensitivity and/or higher background.

1. Perform western transfer as per standard protocols. *See Note F.*
2. Block the membrane in 1x casein solution (10x 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 membrane in unlabeled primary antibody at room temperature with gentle shaking for 30 minutes (or for a time optimized for the concentration of primary antibody used).
4. Wash the membrane 3 times for 4 minutes each in 1x casein solution at room temperature with gentle shaking.
5. Incubate the blot in 1.5 µg/ml of biotinylated secondary antibody in 1x casein solution for 30 minutes at room temperature with gentle shaking. *See Note A.*
6. Wash blot 3 times for 4 minutes each in 1x casein solution at room temperature with gentle shaking.
7. For detecting biotin-labeled secondary antibodies, incubate the blot in 1x casein solution containing one of the following enzyme conjugates for 10-30 minutes:

   VECTASTAIN® ABC-AmP™ (Standard) Kit (Cat. No. AK-6000)
   1 µg/ml AP-streptavidin (Cat. No. SA-5100)
   1 µg/ml AP-anti-biotin (Cat. No. SP-3020)

8. Wash blot 3 times for 4 minutes each in 1x casein solution at room temperature with gentle shaking.
9. Equilibrate blot for 5 minutes in 0.1 M Tris, pH 9.5.
10. Remove excess buffer by holding the blot vertically and touching the edge of the blot to absorbent paper.
11. Place blot target-side-up on plastic wrap on a level surface.
12. Pipet 50 µl/cm² of undiluted DuoLuX™ Chemiluminescent/Fluorescent Substrate onto the blot surface. Incubate for 5 minutes under subdued light.
13. Briefly rinse the blot in 0.1 M Tris buffer, pH 9.5, and remove excess buffer by holding the blot vertically and touching the edge of the blot to absorbent paper.

14a. **Chemiluminescence detection:** Place the blot between two sheets of thin acetate plastic or between layers of plastic wrap and smooth away any bubbles trapped between the layers. Expose to X-ray film or record with a digital imager. The long emission lifetime of the DuoLuX™ Substrate allows the user to re-expose the same blot until optimal signal to noise is achieved.

14b. **Fluorescence detection:** A fluorescent image can be acquired using a digital imaging system or traditional camera with U.V. illumination (254 nm - 365 nm). To enhance fluorescence, expose the blot to U.V. for 2 minutes prior to image acquisition. ***However, U.V. exposure will abolish chemiluminescence,*** so chemiluminescence detection can only be performed prior to fluorescence detection.

## DuoLuX™ chemiluminescence/fluorescence detection protocol for nucleic acid blots:

For Southern and northern applications, DuoLuX™ Chemiluminescent/Fluorescent Substrate performs optimally in conjunction with the reagents shown in the following protocol. These may be obtained individually or as part of the UltraSNAP™ Detection Kit (Cat. No. MB-6500). ***Substitution of these reagents may result in loss of sensitivity and/or higher background.***

1. Perform Southern or northern transfer and hybridization of biotinylated probe using standard protocols (1,2). *See Notes A and E.*
2. Block the blot in Vector® 1x PolyBlock™ Solution for 30 minutes at room temperature with gentle shaking. The volume should be such that the blot is completely covered with blocking solution. *See Note B.*
3. Incubate the blot for 30 minutes with gentle shaking in 1x PolyBlock™ Solution containing 1.0 µg/ml AP-streptavidin (Cat. No. SA-5100) for detecting biotin-labeled probes.
4. Wash the blot 3 times for 10 minutes each in 1x Wash A at room temperature with gentle shaking.
5. Rinse the blot in 1x Wash B.
6. Remove excess Wash B by holding the blot vertically and touching the edge of the blot to absorbent paper.
7. Place the blot target-side-up on plastic wrap on a level surface.
8. Pipet 50 µl/cm² of undiluted DuoLuX™ Substrate onto the blot surface. Incubate for 5 minutes under subdued light.
9. Wash the blot in 1x Wash B for 1 minute at room temperature with gentle shaking. Remove excess liquid from the blot by holding the blot vertically and touching the edge of the blot to absorbent paper ***(do not dry the blot completely)***. *See Note C.*

10a. **Chemiluminescence detection:** Place the blot between two sheets of thin acetate plastic or between layers of plastic wrap and smooth away any bubbles trapped between the layers. Expose to X-ray film or record with a digital imager. *See Note D.*



10b. **Fluorescence detection:** A fluorescent image can be acquired using a digital imaging system or traditional camera with U.V. illumination (254 nm - 365 nm). To enhance fluorescence, expose the blot to U.V. for 2 minutes prior to image acquisition. ***However, U.V. exposure will abolish chemiluminescence,*** so chemiluminescence detection can only be performed prior to fluorescence detection.

## NOTES:

A. Detection of haptens other than biotin (e.g. fluorescein, dinitrophenyl, digoxigenin etc.) can be achieved using the appropriate AP-conjugated antibody for that hapten.

B. PolyBlock™ Solution, Wash A, and Wash B are available in the UltraSNAP™ Accessory Kit (Cat. No. MB-6501).

C. Extensive washing will reduce signal strength; do not extend the wash time unless high background is experienced. If background is excessive, repeat steps 7 through 10 with a wash time of 5-10 minutes in step 9 (optimal wash time is dependent on the degree of background previously detected and, therefore, may require optimization).

D. The long emission lifetime of the DuoLuX™ Substrate allows the user to re-expose the same blot until optimal signal to noise is achieved. Typical exposure times are approximately 5 to 60 minutes when using alkaline phosphatase.

E. Blotting can be done onto either nylon or nitrocellulose. Nylon requires shorter exposure times and is, therefore, preferred for chemiluminescence applications. However, because of nylon's intrinsic fluorescence, nitrocellulose is preferred for fluorescence detection.

F. Nitrocellulose is generally preferred for western blotting applications because of its low background, especially using fluorescent detection. PVDF membranes provide a faster signal development but the background is higher for both chemiluminescence and fluorescence.

**References:**

1. Ausubel FM, R Brent, RE Kingston, DD Moore, JG Seidman, JA Smith, and K Struhl. eds. 1995. Current Protocols in Molecular Biology. John Wiley & Sons, New York, N.Y.
2. Sambrook J, EF Fritsch, and T Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

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For a complete listing of Vector® products and selected protocols visit our website at: **www.vectorlabs.com** and for technical information and support email us at: **vector@vectorlabs.com**