VECTOR LABORATORIES

DuoLuX™

Chemiluminescent/Fluorescent Substrate

for Peroxidase

Cat. No. SK-6604

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, 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. However, AP will provide a higher signal to noise ratio than HRP and is, therefore, recommended for applications where optimal sensitivity is required.

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 HRP is approximately 1 pg of target protein. Film exposure times are generally 5-30 seconds. 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. Sensitivity using HRP is approximately 10 pg with film exposure times from several seconds to a few minutes. Signal can be developed on either nylon or nitrocellulose membranes. *See note E.*

Vector Laboratories, Inc., 30 Ingold Road, Burlingame, CA 94010 U.S.A.
Tel: (650) 697-3600 • Fax: (650) 697-0339 • Email: vector@vectorlabs.com • Web site: www.vectorlabs.com

The DuoLuX™ Chemiluminescent/Fluorescent Substrate for peroxidase (Cat. No. SK-6604) is supplied in two bottles, consisting of the DuoLuX™ Substrate (Reagent **1**) and a peroxidase converter solution (Reagent **2**). To prepare the DuoLuX™ peroxidase substrate, Reagents **1** and **2** are mixed in equal volumes just prior to use. This kit provides 200 ml of substrate working solution.

DuoLuX™ chemiluminescence/fluorescence detection protocol for western blots:

For western and protein dot blot detection, DuoLuX™ Substrate performs optimally using the reagents shown in the following protocol. Use of alternative reagents is possible, but may result in lower sensitivity and/or higher background.

1. Perform western transfer as per standard protocols. We recommend blotting onto PVDF for faster signal development. Nitrocellulose also provides excellent results.
2. Block the membrane in 1x casein solution (10x Casein Solution, Cat. No. SP-5020) or Animal-Free Blocker™ (Cat. No. SP-5030) 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 A.*
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 B.*
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:

   1 µg/ml HRP-streptavidin (Cat. No. SA-5004)
   1 µg/ml HRP-anti-biotin (Cat. No. SP-3010)
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 PBS.
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. Mix an equal volume of DuoLuX™ Chemiluminescent/Fluorescent Substrate (Reagent **1**) with peroxidase converter solution (Reagent **2**) immediately prior to use. Pipet 50 µl/cm² of this 1:1 mixture 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. ***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 B and F.*
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 C.*
3. Incubate the blot for 30 minutes with gentle shaking in 1x PolyBlock™ Solution containing 0.1 μg/ml HRP-streptavidin (Cat. No. SA-5004) 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. Mix an equal volume of DuoLuX™ Chemiluminescent/Fluorescent Substrate (Reagent **1**) with peroxidase converter solution (Reagent **2**) immediately prior to use. Pipet 50 μl/cm^2 of this 1:1 mixture 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 D.*

10.a **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 E.*

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. When using anti-goat or anti-sheep IgG secondary antibodies, the use of bovie products such as casein, serum, albumin or non-fat dry milk as blocking agents may produce high background due to cross-reactivity with bovine immunoglobulins that my be present. In this case, Animal-Free Blocker™ (Cat. No. SP-5030) is recommended.

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

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

D. 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).

E. 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 seconds when using HRP.

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

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.
