VECTOR LABORATORIES

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USER GUIDE

DuoLuX® Chemiluminescent/Fluorescent Substrate

Alkaline Phosphatase (AP)

Cat. No.: SK-6605

Storage: 2-8 °C

DESCRIPTION

DuoLuX Chemiluminescent/Fluorescent Substrate is an acridan-based substrate that offers high sensitivity, versatility, and the convenience of using chemiluminescent or fluorescent visualization methods. It can be used in many protein and nucleic acid detection applications (Southern, northern, western or dot blotting, colony lifts, etc.).

Chemiluminescent properties. Reacted DuoLuX Substrate luminesces in the blue range with a peak emission at 453 nm. Blots can be re-exposed to film as often as necessary over many hours. PVDF, nitrocellulose or nylon membranes can be used, although the chemiluminescent signal develops faster on nylon and PVDF.

Fluorescent properties. Fluorescence can be recorded with a digital imaging system or a conventional camera months after chemiluminescence has faded. The excitation maximum is at 405 nm, but other wavelengths (254 nm and 365 nm) also excite. Maximum fluorescence emission occurs at 453 nm. Acquisition of the fluorescent signal requires a much shorter exposure time than chemiluminescence, often a fraction of a second. PVDF membranes tend to have higher fluorescent background and may not be suitable for fluorescent imaging.

Sensitivity.

Western blot / protein dot blot: Detection to ~1pg of target protein. Film exposure ~1-5 minutes.

Southern / northern blot, plaque and colony screening: Detection to ~ 100fg of DNA/RNA. Film exposure range from 30 seconds to 10 minutes.

KIT COMPONENTS

Product	Volume
DuoLuX Chemiluminescent/Fluorescent Substrate for Alkaline Phosphatase	100 ml

The DuoLuX Chemiluminescent/Fluorescent Substrate for AP is supplied in a ready-to-use form consisting of 100 ml of reagent.

STORAGE

- Store kit at 2-8 °C.

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. ***See Note A.***
2. Block the membrane in blocking solution for 30 minutes at room temperature with gentle shaking. We recommend WestVision Block and DIluent (SP-7000) or 10x Casein (SP-5020) diluted to 1x with deionized water. The volume should be such that the blot is completely covered with blocking solution. For alternative blocking solutions. ***See Note V.***
3. Incubate the membrane in primary antibody diluted in blocking solution at room temperature, with gentle shaking, for 30 minutes (or for a time optimized for the concentration of primary antibody used). Generally, the primary antibody is diluted to between 0.2 – 1 µg/ml or according to manufacturer's recommendations.
4. Wash the membrane 3 times for 5 minutes each in blocking solution or TBST (50 mM Tris, pH 7.6, 150 mM NaCl, and 0.05% Tween® 20) at room temperature with gentle shaking.
5. Incubate the blot for 30 minutes at room temperature with gentle shaking in blocking solution containing 1.0 µg/ml of the appropriate AP conjugate.
6. Wash blot 3 times for 5 minutes each in blocking solution or TBST at room temperature with gentle shaking.
7. Equilibrate blot for 5 minutes in 0.1 M Tris, pH 9.5.
8. Remove excess buffer by holding the blot vertically and touching the edge of the blot to absorbent paper.
9. Place blot target-side-up on plastic wrap on a level surface.
10. Pipet 50 µl/cm^2 of undiluted DuoLuX Substrate onto the blot surface. Incubate for 5 minutes under subdued light.
11. 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 visualization.

See reverse side for additional information.

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30 Ingold Road, Burlingame, CA 94010 | 650.697.3600 p | 650.697.0339 f | vectorlabs.com

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DETECTION PROTOCOL FOR NUCLEIC ACID BLOTS

For Southern and northern applications, DuoLuX 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 labeled probe using standard protocols (1, 2). ***See Note B.***

2. Block the blot in 1x casein 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 blocking solution containing 1.0 µg/ml of the appropriate AP conjugate.

4. Wash the blot 3 times for 10 minutes each in TBST (50 mM Tris, 150 mM NaCl, 0.05% Tween® 20, pH 7.6) at room temperature with gentle shaking.

5. Remove excess TBST by holding the blot vertically and touching the edge of the blot to absorbent paper.

6. Place the blot target-side-up on plastic wrap on a level surface.

7. Pipet 50 µl/cm^2 of undiluted DuoLuX Substrate onto the blot surface. Incubate for 5 minutes under subdued light.

8. 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).

9a. 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.

9b. 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 visualization.

NOTES:

A. Nitrocellulose is generally preferred for western blotting applications because of lower background. For fluorescence visualization, it is important to use a membrane with low autofluorescence. In general, nitrocellulose has lower autofluorescence. Signal development on PVDF membranes is faster, but background may be higher for both chemiluminescence and fluorescence visualization.

B. Blotting can be done onto either nylon or nitrocellulose. Nylon requires shorter exposure times and is, therefore, preferred for chemiluminescence applications. For fluorescence visualization, it is important to use a membrane with low autofluorescence, and in general, nitrocellulose has lower autofluorescence.

C. Other blocking solutions commonly used in blotting applications: (i) 1% BSA (SP-5050) in TBST (50 mM Tris, pH 7.6, 150 mM NaCl, and 0.05% Tween® 20).

or

(ii) BLOTTO (5% non-fat dry milk, 25 mM Tris, pH 7.4, 150 mM NaCl). Casein solution usually results in lower background than other blocking solutions especially with chemiluminescence 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.