USER GUIDE

# DuoLuX® Chemiluminescent/ Fluorescent Substrate

## Peroxidase (HRP)

Together we breakthrough™

**Cat. No.** SK-6604

**Storage** Store kit at 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.

Fluorescent properties. Fluorescence can be recorded with a digital imaging system or a conventional camera months after chemiluminescence has faded. The excitation maximum is 405 nm but other wavelengths (245 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.

Sensitivity
Western blot / protein dot blot: Detection to ~ 1pg of target protein. Film exposure ~5–30 seconds.

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

**Kit Components**

| Product Name | Volume |
|---|---|
| DuoLuX Substrate Reagent 1 | 100 ml |
| DuoLuX HRP Converter Solution Reagent 2 | 100 ml |

The DuoLuX Chemiluminescent/Fluorescent Substrate for HRP is supplied in two bottles, consisting of the DuoLuX Substrate (Reagent 1) and a HRP 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 contains sufficient reagents for 200 ml of substrate working solution.

### 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 WestVision™ Block and Diluent (SP-7000) or Animal-Free Blocker® (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). WestVision Block and Diluent can be used for primary antibody dilution.
4. Wash the membrane 3 times for 4 minutes each in WestVision Block and Diluent solution at room temperature with gentle shaking.

5a. Incubate the blot in 1.5 µg/ml of biotinylated secondary antibody in WestVision Block and Diluent solution for 30 minutes at room temperature with gentle shaking. See Note B.

5b. For direct detection of mouse or rabbit primaries use WestVision Peroxidase Polymer Anti-Mouse IgG (WB-2000) or WestVision Peroxidase Polymer Anti-Rabbit IgG (WB-1000) as instructed.

6. Wash blot 3 times for 4 minutes each in WestVision Block and Diluent solution at room temperature with gentle shaking.
7. For detecting biotin-labeled secondary antibodies, incubate the blot in WestVision Block and Diluent solution diluted into one of the following enzyme conjugates for 10–30 minutes:
   1 µg/ml Streptavidin, HRP (SA-5004)
   1 µg/ml Goat Anti-Biotin, HRP (SP-3010)
8. Wash blot 3 times for 4 minutes each in WestVision Block and Diluent 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 Substrate Reagent 1 with DuoLuX HRP 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.

13a. 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.

13b. 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.

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## Alkaline Phosphatase (AP)

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**Cat. No.** **SK-6604**

### 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 biotinylated probe using standard protocols (1,2). See Notes B and E.
2. Block the blot in WestVision Block and Diluent solution 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 for 30 minutes with gentle shaking in WestVision Block and Diluent solution diluted into 0.1 µg/ml Streptavidin, HRP for detecting biotin-labeled probes.
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. Mix an equal volume of DuoLuX Substrate Reagent 1 with DuoLuX HRP 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.

8a. 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 C.

8b. 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. See Note D.

### Notes

A. When using anti-goat or anti-sheep IgG secondary antibodies, the use of bovine 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 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. 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.

D. Blotting can be done onto PVDF, nylon or nitrocellulose membranes. 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.




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