Lumi-Phos® Plus

Chemiluminescent Reagent

Product Application Instructions

Catalog Number **P-701**

Contents **100 mL single ready-to-use formulation**

Description **Lumi-Phos Plus** reagent is recommended for either direct chemiluminescent detection of alkaline phosphatase (AP) labeled nucleic acids or indirectly to detect hapten labeled nucleic acids probed with specific anti-hapten antibodies conjugated with AP on a Southern blot membrane or any other solid surface. However, it can also be used for the detection of proteins in an ELISA or Western blot membrane using AP- conjugated antibodies specific to the protein of interest.

Note: Lumi-Phos Plus is invented, developed and manufactured by Lumigen.

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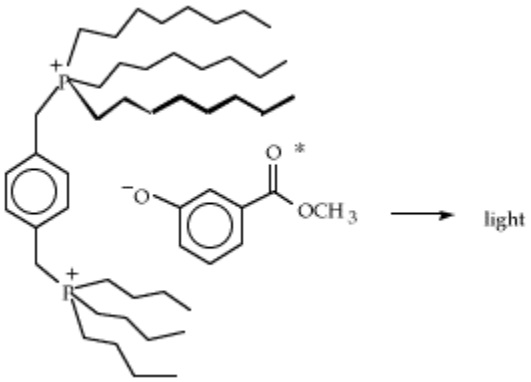
Product Overview

Lumi-Phos Plus is a novel, ready-to-use formulation containing Lumigen PPD (4-methoxy-4-(3-phosphatephenyl) spiro[1,2-dioxetane-3,2'-adamantane], disodium salt) and the patented enhancer shown below in 2-amino-2-methyl-1-propanol buffer (pH 9.6). This formulation provides highly sensitive chemiluminescent detection of alkaline phosphatase with DNA probes or antibody conjugates in solution and on solid supports such as membranes. Light emission is maximal at 470 nm with Lumi-Phos Plus.

Product Structure and Generation of Chemiluminescent Signal



Product Characteristics and Applications

Lumi-Phos Plus provides enhanced chemiluminescence characterized by:

- low background
- fast signal ramp-up
- high intensity light production
- consistent, reproducible reaction in homogeneous aqueous solution and on nylon membranes
- high stability and long shelf life

General Guidelines for the Use of Lumi-Phos Plus

Lumi-Phos Plus performs optimally in alkaline buffer (pH 9.5) at 37°C. At the appropriate concentrations of alkaline phosphatase in solution or on a membrane, the chemiluminescence increases with time and reaches a plateau after approximately 30 minutes. With low concentrations of enzyme, the light intensity remains constant for more than an hour. In the absence of an alkaline phosphatase at 37°C, a small background

chemiluminescent signal may be observed which should reach a constant value as soon as the temperature has stabilized. If it does not remain constant, some source of alkaline phosphatase contamination has occurred. Non-enzymatic chemiluminescence background is linearly related to the amount of Lumi-Phos Plus solution and is also dependent on temperature. Precise temperature control assures uniform chemiluminescence measurements.

Product Handling Instructions

Storage:

Store bottled product at 2 to 8ºC.
Protect from light contamination.
Do not freeze.

Use in subdued light. Indirect incandescent lighting is preferred. Exposure to direct light will cause elevated background.

1) Allow solution to come to room temperature (Approximately 1 hour for 100 mL)
2) Gently invert (4-5 times) the container to mix the reagent.
3) Dispense the amount needed into a separate container. Container should be opaque or covered with aluminum foil to protect from direct light (daylight or artificial).
4) In subdued light, add the solution to microtiter wells or membrane with AP bound protein or nucleic acids.
5) Read chemiluminescent signal on a luminometer or capture the image from membrane on an x-ray film or with CCD camera.

Note: Lumi-Phos Plus can be stored at 25°C for one week with no detectable change in performance. For extended periods, it should be stored at 2-8°C protected from intense light. Under these conditions Lumi-Phos Plus is stable for 2 years. Care should be taken to minimize the chance of contamination with enzyme through handling. Bottles of Lumi-Phos must be completely equilibrated to room temperature before opening.

Repackaging:

Repackaging of Lumi-Phos Plus is discouraged as reliability can be compromised by contamination. Bottling is available from Lumigen to suit the volume demands for your specific work process.
If you choose to repackage, new opaque HDPE or PP plastic containers are required. The high temperatures in the container molding process destroy most or all potential contaminants.

Reusing and washing of containers can lead to contamination and subsequent high background.

Southern Blotting – Equipment and Material Required

1. Depurinating solution – 0.25 M HCl
2. Denaturation solution – 1.5 M NaCl/0.5 M NaOH
3. Neutralization solution – 1.5 M NaCl/0.5 M Tris.Cl, pH 7.0
4. 20X and 2X SSC
5. Tray for transfer buffer
6. Whatman 3MM filter paper
7. Absorbent paper towels
8. Parafilm
9. Glass or plastic plate
10. Nylon or nitrocellulose membranes
11. UV transilluminator or cross linker
12. Plastic sheets or wrap
13. Agarose
14. 1X TAE or 1X TBE buffers
15. Horizontal electrophoresis apparatus
16. Restriction enzymes
17. 1X Wash buffer – 0.1 M Maleic acid, 0.14 M NaCl pH 7.5, 0.3% Tween 20)
18. 2% Blocking buffer – Blocking reagent (Roche Applied Science) in 0.1 M Maleic acid, 0.15 M NaCl, pH 7.5
19. Anti-hapten (biotin or fluorescein or digoxigenin) antibody AP conjugated
20. AP Detection buffer – 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$

Southern Blotting Procedure and Chemiluminescent Detection

Blotting:

1. Digest the DNA with restriction enzyme of choice and electrophorese in an agarose gel in 1X TAE or 1X TBE buffer along with DNA markers (unlabeled or labeled with the same hapten as the one on the hybridization probe to be used) in gel loading dye (bromophenol blue/xylene cyanol).
2. Depurinate the DNA by soaking and gently shaking the gel in 0.25 M HCl on a shaker platform for 20 to 30 min. depending on the thickness and size of the gel.
3. Remove the HCl, rinse the gel with distilled water, and denature DNA by soaking/shaking in denaturation solution for 20 min. Remove the denaturation solution and repeat the denaturation with fresh solution depending on the size and thickness of the gel.
4. Remove the denaturation solution, rinse the gel with distilled water, and neutralize the gel by soaking/shaking in neutralization solution for 20 to 30 min.

5. Blot the DNA from the gel onto Nitrocellulose or nylon membrane by capillary transfer using 20X SSC by sponge method or Whatman 3MM filter paper wick method (see the set up in Current Protocols in Molecular Biology, eds. FM Ausubel et al., Volume 1, Section IV, Unit 2.9A).
6. Remove the membrane from the transfer stack, rinse in 20X SSC and tightly bind the DNA to the membrane either by baking under vacuum for 2hrs at 80^{o}C for nitrocellulose or by UV cross linking on a transilluminator for nylon membrane.

Hybridization:

1. Prehybridize the DNA blot for 2 to 3 hr. with hybridization solution which can be aqueous or containing formamide. Preybridization and hybridization are done at about 65^{o}C or 42^{o}C depending on the type of hybridization solution and the size of DNA probe used. For hybridization solution recipes see Current Protocols in Molecular Biology, eds. FM Ausubel et al., Volume 1, Section IV, Unit 2.10.
2. Remove the prehybridization solution and hybridize the blot with denatured (heated at 95^{o}C for 5 min. and quick cooled on ice) labeled DNA probe in fresh hybridization solution overnight in a shaking or rotating incubator set at the desired temperature. DNA probes can be labeled with haptens such as biotin, fluorescien or digoxigenin for which antibodies are commercially available. The amount of DNA probe needed depends on the probe length and the amount of hybridization buffer used. An amount between 1 and 20ng of probe per mL of hybridization solution is normally sufficient.
3. Remove the hybridization solution and wash with 2x SSC/0.1% SDS for 15 min. Wash temperature depends on the desired washing stringency.
4. Repeat the wash with 0.2x SSC/0.1% SDS for 15 min.
5. If desired, wash again with 0.1X SSC/0.1% SDS for 15 min.

Detection:

(All operations are performed at room temperature)

1. Wash the blot in 1X wash buffer for 15 min.
2. Remove the wash buffer and block the blot in 2% blocking buffer for 1 hr.
3. Remove the blocking buffer and incubate the blot for 1 hr. in alkaline phosphatase (AP) conjugated anti-hapten antibody diluted in 2% blocking buffer.
4. Remove the antibody from the blot and wash 2X for 20 min. each in 1X wash buffer.
5. Rinse the blot in AP detection buffer for 5 min.
6. Take the blot into dark room and add Lumi-Phos Plus substrate to cover the blot and wait for 5 min.
7. Hold the blot with forceps to let the excess substrate drip on to a Kim-wipe and place the blot on a plastic sheet. Cover the blot with another plastic sheet and wipe away excess substrate from the blot.

8. Expose the blot to an X-ray film to desired amount of time to be able to detect the DNA bands.

Troubleshooting Tips for Southern Blotting and Detection

There are mainly two types of problems associated with the detection of DNA on Southern blot.

1. **Signal problems:** Very high, weak and no signal
2. **Background problems:** High non-specific background

Signal Problems	Possible Causes	Troubleshooting Tips
1. Weak signal	• Low concentrations of AP detection antibody • Poor DNA – antibody binding • Poor transfer of DNA to the blot • Inhibition of AP enzyme by components in wash and blocking buffers • Expired or contaminated APS-5 substrate	• Use higher concentrations of AP detection antibody • Use highly specific antibody • Check the DNA transfer set up • Check the components in blocking and wash buffers and replace them with new buffers • Use a new lot of substrate
2. Very high signal	• High concentrations of antibody	• Titrate the antibody
3. High signal followed by fast signal decay	• Very high concentration of AP antibody	• Use more diluted AP antibody
4. No signal	• Lack of antibody binding • Inactive AP enzyme • Inhibition of AP enzyme by components in wash and blocking buffers • Expired substrate	• Replace with highly specific antibodies • Use a new lot of AP detection antibody • Check the components in blocking and wash buffers and replace them with new buffers • Use a new lot of substrate

Background Problems	Possible Causes	Troubleshooting Tips
1. Very high background on the blot	• Use of high amount of AP antibody • Insufficient washing of the blot • Inadequate blocking	• Use more diluted AP detection antibody • Increase number of washes • Block longer period of time or change

		blocking agent
2. Higher than normal Lumi-Phos Plus substrate background	• Contaminated substrate	• Use a new lot of substrate

Lumigen Product Matrix

	Lumigen ECL Plus	Lumigen ECL Extra	Lumigen ECL Ultra	Lumi-Phos Plus	Lumi-Phos 530	Lumigen APS-5	Lumi-Phos HRP	Lumigen SPARCL	Lumigen HyPerBlu
Application	Western Blotting	Western Blotting	Western Blotting	Southern Northern Blotting	ELISA	ELISA	ELISA	ELISA, Other Proximity Assays	H_2O_2 Detection
Enzyme	HRP	HRP	HRP	AP	AP	AP	HRP	HRP	Direct, Non-enzymatic
Sensitivity	Low pg to mid fg	Low pg to mid fg	Mid to low fg	Single Copy Gene Detection	Low pg to fg	Low pg to fg	Low pg to fg	Low pg	2.6 nM
Signal Duration	Up to 5 Hours	Up to 6 Hours	Up to 8 Hours	Up to 24 Hours	Up to 24 Hours	Up to 4 Hours	Up to 5 Hours	Instantaneous Flash	Up to 8 Hours
Shelf Life	2 Years	1 Year	1 Year	2 Years	2 Years	1 Year	1 Year	1 Year	2 Years
Storage Conditions	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C

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