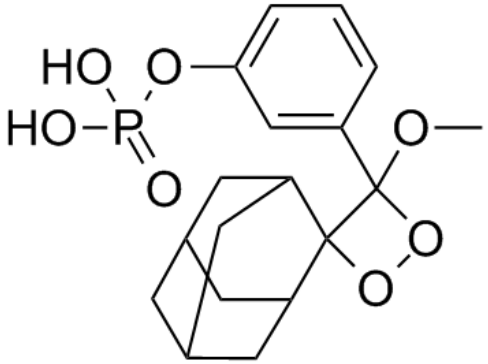
Product Data Sheet

Product Name: AMPPD (Lumi-Phos Plus)
Cat. No.: GC30019

Chemical Properties

Cas. No.	122341-56-4		
SMILES	COC1(C2=CC(OP(O)(O)=O)=CC=C2)C3(C(C4)CC5CC4CC3C5)OO1		
Formula	$C_{18}H_{23}O_7P$	M.Wt	382.34
Solubility	Water : ≥ 125 mg/mL (326.93 mM)	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Structure



Protocol

Alkaline Phosphatase Activity Assay

This protocol is adapted from research data and provided for reference only. Adjustments may be necessary based on specific experimental requirements.

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(1) Enzyme Sample Preparation: Preserve samples containing alkaline phosphatase (e.g., cell lysate supernatant or immunodetection complexes) in 50mM carbonate buffer (pH=9.5) at 4°C for later use.

(2) Alkaline Phosphatase Activity Detection: The reaction system contains 0.0125–0.4mM AMPPD, 50 mM carbonate buffer (pH=9.5), and 1mM $MgCl_2$, with the total volume set according to the experiment. Incubate at room temperature for 20 minutes before detection.

(3) Photometer Detection: Use a luminometer to measure the chemiluminescent signal, recording either the average or integrated light intensity over 10 or 30 seconds.

(4) Film Imaging Detection: For alkaline phosphatase immobilized on membranes (e.g., Western blot or dot blot), incubate the membrane with AMPPD substrate and then expose to photographic film for imaging.

Precautions:

1) The optimal pH for the chemiluminescent reaction is 9.0–9.5, where the signal-to-noise ratio (enzymatic reaction/non-enzymatic hydrolysis) is highest.

2) For your safety and health, please wear a lab coat and disposable gloves when operating.

References:
[1] Bronstein I, Edwards B, Voyta JC. 1,2-dioxetanes: novel chemiluminescent enzyme substrates. Applications to immunoassays. J Biolumin Chemilumin. 1989 Jul;4(1):99-111.

Background

AMPPD (Lumi-Phos Plus) is a 1,2-dioxetane derivative that serves as an ultrasensitive chemiluminescent substrate for alkaline phosphatase and is widely used in biochemical detection [1-2]. Under the catalysis of alkaline phosphatase, AMPPD undergoes dephosphorylation to form an unstable intermediate. This intermediate decomposes, leading to cleavage of the dioxetane structure and emission of light at a wavelength of 470nm, with the luminescence intensity being proportional to the concentration of alkaline phosphatase[3-4].

References:
[1] Yue L, Liu YJ. Mechanism of AMPPD Chemiluminescence in a Different Voice. J Chem Theory Comput. 2013 May 14;9(5):2300-12.

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[2] Tu L, Wang Y, Yang Y, et al. Spectroscopic study of the authentic emitter of AMPPD chemiluminescence in alkaline aqueous solution. Phys Chem Chem Phys. 2010 Jul 7;12(25):6789-94.
[3] Cate RL, Ehrenfels CW, Wysk M, et al. Genomic Southern analysis with alkaline-phosphatase-conjugated oligonucleotide probes and the chemiluminescent substrate AMPPD. Genet Anal Tech Appl. 1991 May;8(3):102-6.
[3] Cate RL, Ehrenfels CW, Wysk M, et al. Genomic Southern analysis with alkaline-phosphatase-conjugated oligonucleotide probes and the chemiluminescent substrate AMPPD. Genet Anal Tech Appl. 1991 May;8(3):102-6.
[4] Hai Z, Li J, Wu J,, et al. Alkaline Phosphatase-Triggered Simultaneous Hydrogelation and Chemiluminescence. J Am Chem Soc. 2017 Jan 25;139(3):1041-1044.

Caution: Product has not been fully validated for medical applications. For research use only.
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