

## 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

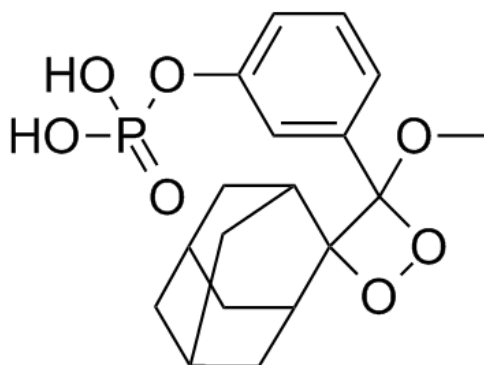
Formula  $C_{18}H_{23}O_7P$  M.Wt 382.34

Solubility Water :  $\geq 125$  mg/mL (326.93 mM) Storage Store at  $-20^{\circ}\text{C}$

General tips For obtaining a higher solubility , please warm the tube at  $37^{\circ}\text{C}$  and shake it in the ultrasonic bath for a while. Stock solution can be stored below  $-20^{\circ}\text{C}$  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.

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

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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<sub>2</sub>, 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.

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