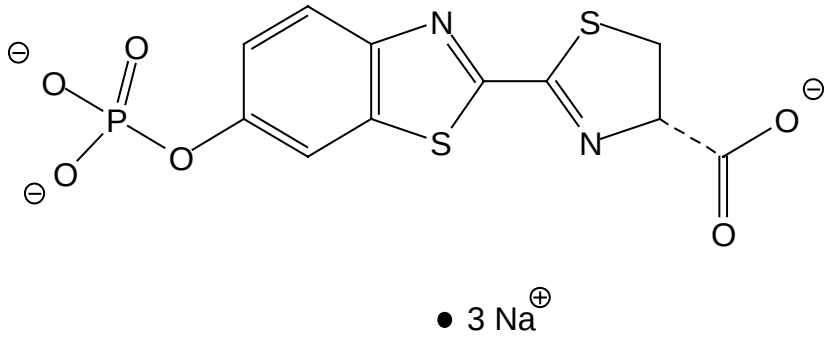
**ProductInformation**

**D-LUCIFERIN 6-O-PHOSPHATE SODIUM**
**Sigma Prod. No. L9402**



• 3 $Na^{\oplus}$

**CAS NUMBER:** 145613-12-3
**SYNONYM:** 4,5-Dihydro-2-(6-phospho-2-benzothiazolyl)-4-thiazolinecarboxylic acid, sodium salt

**PHYSICAL DESCRIPTION:**

Appearance: (Lyophilized) light yellow powder with very faint green cast
Molecular formula: $C_{11}H_6N_2O_6S_2PNa_3$
Formula weight: 426.2
Spectral data: excitation at 345 nm, emission at 442 nm
(aqueous buffer, pH 9.8)[1]
Derived $E^{M}_{311\ nm}$ = 15,500 (correcting for complexed water, in 0.1 M HCl).[1,2]

**STORAGE / STABILITY AS SUPPLIED:**

The dry solid is stable frozen at least two years at -20°C.

**SOLUBILITY / SOLUTION STABILITY:**

The contents of a 1 mg vial will dissolve in 0.1 mL water to give a clear yellow solution. One mg will yield approx. 120 mL of 0.02 mM solution. Store working solutions light-protected on wet ice before use. General recommendation for fluorescent substrates is to freeze solutions in working aliquots and store protected from light.

**GENERAL REMARKS:**

Conjugation of luciferin with phosphate makes a phosphate ester at the 6-hydroxy position, which is not recognized by luciferase. However, if the phosphate ester bond is cleaved with alkaline phosphatase, luciferase will then be able to recognize the liberated native substrate and light will be emitted. Therefore, luciferin-6-O-phosphate can effectively function as a marker for alkaline phosphatase in ultra-sensitive enzymatic assays.

Luciferin phosphate as an alkaline phosphate substrate may be used as follows:[1]
In the first reaction, Luciferin phosphate is converted by AP to free luciferin which is monitored by fluorescence . The Km value was determined.

A sample of 0.40 mL of 0.01 M diethanolamine buffer containing 0.5 M $MgC1_2$, pH 9.8, and 0.05 mL alkaline phosphatase (0.01 U) was incubated for 5 minutes at 37°C. Then 0.05 mL substrate solution at various concentrations (L9402) was added and the decrease in RFU (relative fluorescence units) was measured using excitation at 345 nm, emission at 442 nm (to determine kinetic constants).

In the second reaction Luciferase is measured by light emission in the presence of a constant amount of ATP and luciferase. Then for this assay, using a total volume of 0.5 mL, 0.4 mL of the same buffer with 0.05 mL substrate solution (0.1 mM L9402 in diethanolamine buffer) was incubated for 5 minutes. Then 0.05 mL enzyme solution (as above) was added and incubated for 30 min. Then 0.1 mL of test solution was transferred to 0.4 mL test buffer 30 mM HEPES, 6.6 $MgCl_2$, 0.6 mM EDTA, 0.1 mM DTT, 5 mM ATP, 1 μ luciferase, pH 7.75 and light measured. The sensitivity was reported as approx. 0.05 picograms enzyme per test sample.[1]

Sigma has tested this compound for sensitivity levels of bioluminescence as follows:[2]

- Reagent A: "Basic cocktail": luciferase at 0.01 mg/mL, 265 mM tricine, 5 mM $MgSO_4$, 0.5 mM EDTA, and 0.5 mM dithiothreitol, pH 7.8.
- Reagent B: ATP Stock solution: 23 mg A2343 in 250 mL water, giving 140 μM (140 pmol/μL) - frozen in aliquots.
- Reagent C: 15 mM luciferin-6-O-phosphate (Add 6.4 mg per mL water, stir 5 minutes, protecting from light. Remove 0.1 mL and store separately on ice. To the remaining sample of 15 mM L9402, add alkaline phosphatase (P6772) to 30 units/mL. Stir for 5 minutes, then store on ice.
- Reagent D: Dilute the ATP stock to 0.5 pmol/μL with water (1:280).
- Reagent E: 2 mL Basic cocktail A + 20 μL 0.5 pmol/μL ATP. Store on ice.

Procedure: Aliquot 100 mL reagent E per culture tube into 6 tubes. One tube at a time, add 15 mM L9402 - 2 μL, 4 μL, 5 μL, 6 μL, 8 μL, and 10 μL. Read sample on photometer immediately after addition of luciferin and brief vortexing.

**REFERENCES:**


1. Miska, W. and Geiger, R. *Biol. Chem. Hoppe-Seyler*, 369, 407-411 (1988).
2. Sigma production/quality control.
3. *J. Clin. Chem. Clin. Biochem.*, 27, 361 (1989).