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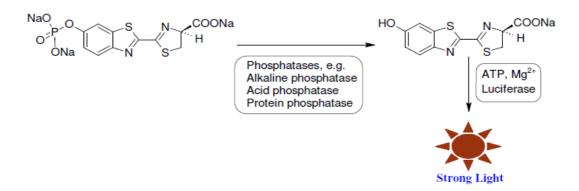
D-Luciferin Phosphate

Product Description

Name :	D-Luciferin phosphate 4,5-Dihydro-2-(6-phospho-2-benzothiazolyl)- 4-thiazolinecarboxylic acid, sodium salt
Catalog Number :	FP-DT244A 1 mg
Structure :	$C_{11}H_6N_2O_6S_2PNa_3$ CAS: [145613-12-3]
Molecular Weight :	MW= 426,25
Solubility:	Water, DMSO
Absorption / Emission :	$\lambda_{exc} \mid \lambda_{em}$ (aqueous buffer, pH 9.8) = 345 / 442 nm
EC (M^{-1} cm ⁻¹):	15,500(correcting for complexed water, in 0.1 M HCl).

Storage: -20°C (Expiration date is 12 months from the date of receipt) Protect from light and moisture

Introduction



D-Luciferin phosphate is a highly sensitive bioluminescent substrate for detecting alkaline phosphatases, acid phosphatases and protein phosphatases by luminometric detection. It is used in combination with luciferase/ATP system. As shown above D-Luciferin phosphate can be converted to D-Luciferin by phosphatases (such as alkaline phosphatases, acid phosphatases and protein phosphatases) that gives luminescence in the presence of luciferase and its cofactors.

Compared to the colorimetric phosphatase assays (such as pNPP) and fluorimetric assays (such as MUP and fluorescein diphosphate), luciferin phosphate provides the phophatase assays with the lowest background and highest sensitivity. For your convenience, you might also consider to use our luciferase assay kit that provides all the essential components for detecting D-luciferin release from D-luciferin phosphate by phosphatases.

Directions for use

Handling and Storage

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.

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FT-DT244A

General recommendation for fluorescent substrates is to freeze solutions in working aliquots and store protected from light.

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Guidelines for use

Km value determination by fluorescence

- Incubate a sample of 0.40 mL of 0.01 M diethanolamine buffer containing 0.5 M MgC12, pH 9.8, and 0.05 mL alkaline phosphatase (0.01 U) for 5 minutes at 37°C.
- Add 0.05 mL substrate solution at various concentrations
- Measure the decrease in RFU (relative fluorescence units) using excitation at 345 nm, emission at 442 nm (to determine kinetic constants).

Luciferase assay by light emission in the presence of a constant amount of ATP and luciferase

- Incubate for 5 minutes in a total volume of 0.5 mL, 0.4 mL of the same buffer with 0.05 mL substrate solution (0.1 mM FP-DT244A in diethanolamine buffer)
- Add 0.05 mL enzyme solution (as above)
- Incubate for 30 min
- Transferre 0.1 mL of test solution to 0.4 mL test buffer 30 mM HEPES, 6.6 MgCl₂, 0.6 mM EDTA, 0.1 mM DTT, 5 mM ATP,1 μg luciferase, pH 7.75
- Measure light

References

- Miska W., Geiger R. Biol. Chem. Hoppe Seyler (1988) 369:407-411
- **Riahi-Madvar A., Hosseinkhani S.,** Design and characterization of novel trypsin-resistant firefly luciferases by sitedirected mutagenesis, *Protein Engineering Design and Selection* 22(11):655-663 (2009) <u>Abstract</u>

Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.

• Luciferase, <u>FP-D1826B</u>

ATP, <u>00064A</u>

Ordering information

<u>Catalog size quantities and prices may be found at www.interchim.com/</u> Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes[®] / Interchim; Hotline : +33(0)4 70 03 73 06

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