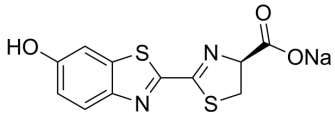
D-Luciferin sodium

Cat. No.:	HY-12591
CAS No.:	103404-75-7
Molecular Formula:	$C_{11}H_7N_2NaO_3S_2$
Molecular Weight:	302.3
Target:	Fluorescent Dye
Pathway:	Others
Storage:	4°C, sealed storage, away from moisture and light * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light)



SOLVENT & SOLUBILITY

In Vitro

H_2O : 250 mg/mL (826.99 mM; ultrasonic and warming and heat to 60°C)
DMSO : 100 mg/mL (330.80 mM; Need ultrasonic)

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	3.3080 mL	16.5399 mL	33.0797 mL
	5 mM	0.6616 mL	3.3080 mL	6.6159 mL
	10 mM	0.3308 mL	1.6540 mL	3.3080 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: PBS
   Solubility: 100 mg/mL (330.80 mM); Clear solution; Need ultrasonic
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (8.27 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (8.27 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

D-luciferin is the natural substrate of the enzyme luciferase (Luc) that catalyzes the production of the typical yellowgreen light of fireflies. The 560 nm chemiluminescence from this reaction peaks within seconds, with light output that is proportional to luciferase concentration when the substrate luciferin is present in excess. The luciferase (luc) gene is a popular reporter gene for research and agent screening. Chemiluminescent techniques are virtually background-free, making the luc reporter gene ideal for detecting low-level gene expression. As little as 0.02 pg of luciferase can be reliably measured in a standard scintillation counter. In addition to its role as a reporter of gene expression, luciferase is commonly used in an extremely sensitive assay for ATP[1]. We of er the firefly luciferase (HY-P1004), luciferin free acid (HY-12591A), as

	well as its water-soluble sodium salts (HY-12591) and potassium salts (HY-12591B) .
In Vitro	1. Precautions a) The D-luciferin salts are readily soluble in aqueous buffers (pH 6.1-6.5) up to 100 mM. Stock solutions can be made in ATP-free water and stored at -20°C, protect from light. The free acid must be neutralized with an appropriate base to solubilize. At a higher pH, luciferin undergoes a base-catalyzed formation of dehydroluciferin, as well as racemization to the L-isomer. b) The D-luciferin can be used with any existing reporter assay or ATP assay system. c) If testing for ATP, minimize all possible sources of ATP contamination by wearing gloves and using ATP-free containers. Use only sterile ATP-free water and reagents. Use autoclaved water for all reagent preparations. 2. Experimental Protocols This protocol only provides a guideline, and should be modified according to your specific needs. The following protocol is an example for potassium and sodium salt preparation. It can be adapted for most cell types and in vivo animal use. 2.1 Example protocol for in vitro bioluminescent image assays a) Prepare a 100 mM (100-200X) Luciferin stock solution in sterile water. Mix well. Use immediately, or make single use aliquots, and store at -20°C, avoid freeze-thaw cycles, avoid exposure to the light. b) Prepare a 0.5-1 mM working solution of D-Luciferin in pre-warmed tissue culture medium. c) Aspirate media from cultured cells. d) Add Luciferin working solution to cells, and incubate the cells for 5-10 minutes at 37°C just prior to imaging. 2.2 Example protocol for in vivo bioluminescent image assays a) Prepare a 15 mg/mL Luciferin stock solution in DPBS, without Mg^{2+} and Ca^{2+}. Mix well. b) Filter sterilizes the solution through a 0.2 µm filter. Use immediately, or make single use aliquots, and store at -20°C, avoid freeze-thaw cycles, avoid exposure to the light. c) Inject the luciferin intra-peritoneally (i.p.) 10-15 minutes before imaging at 150 mg/kg (or 10 µL/g of luciferin stock solution) of the animal body weight. Note: A kinetic study of luciferin should be performed for each animal model to determine peak signal time. 2.3 Example protocol for luciferin reporter assays a) Prepare a 100 mM Luciferin stock solution in sterile water. Use immediately, or make single use aliquots, and store at -20°C, avoid freeze-thaw cycles, avoid exposure to the light. b) Prepare a 1 mM working solution of D-Luciferin with 3 mM ATP, 1 mM DTT and 15 mM MgSO4 in 25 mM tricine buffer pH 7.8. c) Pipette 5-10 µLof cell lysate into a microplate. Use lysis reagent or buffer without lysate as a blank. d) Prime luminometer with luciferin working solution according to manufacturer's instructions. e) Inject 200 µL of luciferin working solution with no delay and a 10 second integration time. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Bioluminescence imaging (BLI) using the firefly luciferase (Fluc) as a reporter gene and D-luciferin as a substrate is currently the most widely employed technique. The total signal intensity is plotted against the time after D-luciferin injection to generate a time-intensity curve. In addition to the peak signal, the signals at fixed time points (5, 10, 15, and 20 min) after D-luciferin injection are determined as alternatives to the peak signal. The signal in a given time-intensity curve is normalized for the peak signal in the curve to represent the pattern of temporal changes after D-luciferin injection[3]. Inject with 10 µL of D-luciferin (intraperitoneally or intravenously) stock solution per gram of body weight: normally ~200 µL for a 20 g mouse for a standard 150 mg/kg injection. Thaw D-Luciferin (either Potassium or Sodium Salt) at room temperature and dissolve in dPBS (no calcium or magnesium) to a final concentration of 15 mg/mL. Pre-wet a 0.22 µm filter by drawing through 5-10 mL of sterile H_2O and discard water. Sterilize the D-Luciferin solution through the prepared 0.22 µm syringe filter. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Animal Administration [3]	Mice[3] In vivo BLI is performed using a cooled charge-coupled device camera system (IVIS Imaging System 100) 3, 5, 7, 10, 12, 14,

19, 21, 24, and 28 days after the inoculation of HCT116-Luc cells. Mice are injected with 75 mg/kg D-luciferin in 100 µL of phosphate-buffered saline subcutaneously near the scapula and were placed in the light-tight chamber of the imaging system. Beginning 5 min after injection, dorsal luminescent images with an exposure time of 1 s are acquired sequentially at a rate of one image per min until 20 min after D-luciferin injection. Data acquisition is continued until 40 min postinjection on days 3 or 5 and until 25 min on day 7, because of the prolonged time course of light emission. Binning is 4 and the field of view is 15 cm.

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CUSTOMER VALIDATION

- Cell Metab. 2022 Sep 7;S1550-4131(22)00359-X.
- Mil Med Res. 2023 Jul 25;10(1):34.
- Adv Funct Mater. 2023 Sep 15.
- Acta Pharm Sin B. 2023 Sep 1.
- Acta Pharm Sin B. 12 March 2022.

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REFERENCES

[1]. Giuseppe Meroni, et al. D-Luciferin, derivatives and analogues: synthesis and in vitro/in vivo luciferase-catalyzed bioluminescent activity. ARKIVOC 2009 (i) 265-288.

[2]. Inoue Y, et al. Timing of imaging after d-luciferin injection affects the longitudinal assessment of tumor growthusing in vivo bioluminescence imaging. Int J Biomed Imaging. 2010;2010:471408.

[3]. Rajesh Shinde, et al. Luciferin derivatives for enhanced in vitro and in vivo bioluminescence assays. Biochemistry. 2006 Sep 19;45(37):11103-12.

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

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