D-Luciferin

Cat. No.: HY-12591A

CAS No.: 2591-17-5

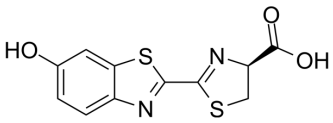
Molecular Formula: $C_{11}H_8N_2O_3S_2$

Molecular Weight: 280.32

Target: Fluorescent Dye

Pathway: Others

Storage: 4°C, protect from light
* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 125 mg/mL (445.92 mM; Need ultrasonic)
H_2O : 9.09 mg/mL (32.43 mM; ultrasonic and adjust pH to 9 with 1M NaOH)
H_2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	3.5674 mL	17.8368 mL	35.6735 mL
	5 mM	0.7135 mL	3.5674 mL	7.1347 mL
	10 mM	0.3567 mL	1.7837 mL	3.5674 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (8.92 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (7.42 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (7.42 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) There are three forms of D-luciferin, namely free acid (HY-12591A), potassium salt (HY-12591B) and sodium salt (HY-12591). The sodium salt and potassium salt forms of D-luciferin are easily soluble in aqueous buffers (pH 6.1-6.5). Stock solutions can be made in ATP-free water and stored at -20□, protect from light. The free acid can be neutralized with DMSO or an appropriate base to dissolve. 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. 2.1 Example protocol for in vitro bioluminescent image assays a) Prepare D-luciferin stock solution in DMSO. Mix well. Use immediately, or make single use aliquots, and store at -20□, 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 D-Luciferin working solution to cells, and incubate the cells for 5-10 minutes at 37□ just prior to imaging. 2.2 In vivo assays Note: Since the in vivo experimental dose is relatively large, generally 150 mg/kg, it is recommended to choose HY-12591B or HY-12591. 2.3 Example protocol for D-Luciferin reporter assays a) Prepare D-luciferin stock solution in DMSO. Use immediately, or make single use aliquots, and store at -20□, 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 D-Luciferin working solution according to manufacturer's instructions. e) Inject 200 µL of D-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.

PROTOCOL

Animal Administration [2]	Mice[2] 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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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.

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