

Luciferins

Product Information

Product name cat.number	MW (g·mol ⁻¹)	$\lambda_{exc}/\lambda_{em}$ (nm)	mol. abs. (M ⁻¹ cm ⁻¹)	Solubility	Comments
D-Luciferin, free acid FP-27060A, 10 mg FP-270609, 25 mg FP-27060B, 250 mg FP-27060D, 1 g	280.33	328 / 532	18 000	DMSO Not soluble in neutral buffers	Make your buffers alkaline prior to dissolving the D-Luciferin Free Acid, then titrate to your desired pH.
D-Luciferin, Na salt FP-72604A, 25 mg FP-72604B, 50 mg FP-72604C, 1 g	302.30	328 / 533	17 000	water pH>6	
D-Luciferin, K salt FP-M1224A, 25 mg FP-M1224B, 50 mg FP-M1224C, 500 mg FP-M1224D, 1 g	318.42	328 / 533	17 000	water pH>6	Recommended salt form for <i>in vivo</i>
D-Luciferin, Ethyl ester FP-CF4420, 2 mg FP-CF4421, 10 mg	306.36				A membrane permeable analog of the primary substrate for the firefly luciferase light producing system with 30% higher light intensity
D-Luciferin, 6-methyl ester FP-M1418A, 10 mg	294.3				An inhibitor of firefly luciferase light producing system. Useful in chemiluminescent dual assay systems with both luciferase and cytochrome P450 enzymes

Storage: -20°C >1 year. (M)
Long term storage at -70°C

Protect from light and moisture

Introduction

The highly purified synthetic luciferin exhibits physical properties identical to those of natural luciferin, isolated from fireflies (*Photinus pyralis*) and other beetles. Enzymatic activity is optimal.

Luciferin is a generic term for photon emitting biomolecules, well represented in several marine species, bacteria, protistes, fishes, insects¹.

D-Luciferin is a luminescent substrate for firefly luciferase, a monomeric 61kDa protein (encoded by luc gene), generating a green light flash. It is primarily used in reporter assays and ATP assays. Its bioluminescent reaction is the most efficient known in nature! (With about 90 % of the energy released being converted to light): ATP-dependent oxidation of luciferin by luciferase results in bioluminescence ($E_m = 560$ nm) that is longer and brighter in presence of CoenzymeA, at neutral and alkaline pH. Bioluminescence is red-shifted ($E_m = 617$ nm) under acidic conditions. Optimized formulations allow linear results over >8 orders of magnitude of enzyme concentration, down less than 10–20 moles of luciferase.

The luciferin/luciferase system is used as a very sensitive reporter assay for gene expression in plants, bacteria, mammalian cells, and for monitoring baculovirus gene expression in insects. It can be used too for ATP assays in research applications or for detect bacterial contamination detection (used to measure 10⁻¹⁵ molar quantity of ATP). It also has been used for detecting certain amphipathic and hydrophobic substances, including anesthetics and hormones, as these compete with luciferin for the hydrophobic site on the luciferase molecule (*Anal Biochem* 190, 304 (1990)).

Production of light can be monitored with either a luminometer, including multiwell plate automatized instruments, or a scintillation counter.

Directions for use

Handling and Storage

Luciferin free acid is soluble in DMSO at pH>6 but may be dissolved directly in Tricine or HEPES buffer (max. 1.5~mM). Potassium salt and Sodium salt are soluble in water or aqueous buffer up to 100mM at pH>6.

Protocol 1: Luciferin Reporter Assay

- 1- Dissolve 1 mM luciferin or luciferin salt, 3 mM ATP, Triton-X 100 (1%), 25 mM glycylglycine, PH 7.8, 15 mM MgSO₄, 4 mM EGTA and 1 mM DTT in fresh desionized ATP free water

Note: The Luciferin concentration can be checked by absorbance measurement at 385nm in 0.5 M carbonate buffer, pH 11.5. Molar extinction is 18 000 M⁻¹cm⁻¹.

- 2- Warm luciferin substrate reagent to room temperature before starting.
- 3- Lyse cells using luciferin substrate reagent.
- 4- Pipet 5-10 µl of cell lysate into a microplate. Use buffer without cells as blank.
- 5- Prime luminometer with luciferin substrate solution according to manufacturer's instructions.
- 6- Set luminometer to inject 200 µl of Luciferin Substrate with no delay and a 10 -second integration time.

Notes:

- The numerical instrument results (RLUs) for a given sample or standard will vary from day to day. However, the relative differences between samples or standards should be consistent.
- If testing for ATP minimize all possible sources of ATP contamination by wearing gloves and using only ATP-free containers. Use only sterile ATP-free water and reagents (autoclave water and use autoclaved water for all reagent prep).
- Sanitize luminometer injector lines each day before running samples. Use 1% bleach or other sterilant. Ask luminometer manufacturer for appropriate solution for their instrument.
- Store any substrate or samples containing ATP in polypropylene or glass only. Avoid polystyrene.
- Purified luciferase may be used as a positive control.

Here are proven buffer systems that are known maximize performance and sensitivity:

Notes:

To dissolve in buffered saline start by using the predicted molar alkalinity using Sodium Hydroxide (NaOH) added to the buffer before adding d-Luciferin. If a precipitate forms adjust the pH higher until the solution is clear.

Protocol 2: Assay-Reagents for Firefly Glow Luminescence

- 20 mM Tricine
- 1.07 mM (MgCO₃)₄ · Mg(OH)₂ · 5H₂O
- 2.67 mM MgSO₄
- 0.1 mM Ethylenediamine tetra-acetic acid (EDTA)
- 33.3 mM Dithiothreitol (DTT)
- 270 µM Coenzyme A
- 470 µM D-Luciferin
- 530 µM Adenosine Triphosphate (ATP)

* Adjust pH to 7.8 Protect from light, may store at -70° C

Protocol 3: Assay-Buffer for the measurement of ATP using Firefly Luciferase

- 300 µM D-Luciferin
- 5 µg protein/ml Firefly Luciferase
- 75 µM Dithiothreitol (DTT)
- 25 mM HEPES
- 6.25 mM MgCl₂
- 0.63 mM Ethylenediamine tetra-acetic acid (EDTA)
- 1.0 mg Protein/ml Bovine Serum Albumin (BSA)

*Adjust to pH 7.8 Protect from light, may store at -70° C

FT-27060A

Apply Firefly Luciferase immediately before measurement starts

All liquids that used for in vivo tests and experiments, we recommended make a sterile filtration, please filtrate through 0.25 µm filter.

Other protocol may found in the literature.

Related products

- Firefly Luciferase, recombinant, [FP-D1826B](#)
- ATP disodium salt, [00064A](#)
- Luciferase reporter gene assay kit, [FP-JQ6810](#)
- DMNPE-caged luciferin (cross easily biological membranes), [FP-21639A](#)
- Firefly & Renilla Luciferase Assay kit, [FP-BE7810](#)
- Coelenterazine native, [UP972333](#)
- Coelenterazine H, [UPR30783](#)

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