

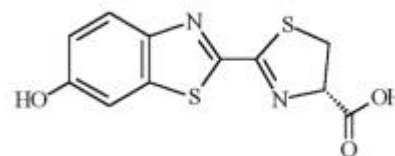
# Luciferins

## Product Description

*D-Luciferin is primarily used in reporter assays and ATP assays*

**Catalog number:** [27060A](#)  
**Name:** **D-Luciferin, free acid**  
**Formula:** (S)-4,5-Dihydro-2-(6-hydroxy-2-benzothiazolyl)-4-thiazolecarboxylic acid  
 CAS[2591-17-5], C<sub>11</sub>H<sub>8</sub>N<sub>2</sub>O<sub>3</sub>S<sub>2</sub>, M.W.= 280

White solid soluble in DMSO and slightly soluble in water.  
 Purity: >99%



**Catalog number:** [M1224A](#)  
**Name:** **D-Luciferin, K salt**  
**Formula:** CAS[ 115144-35-9], C<sub>11</sub>H<sub>7</sub>KN<sub>2</sub>O<sub>3</sub>S<sub>3</sub>, M.W.= 318.42  
 Light yellow solid , water-soluble form of D-luciferin  
 Purity: >99%

**Catalog number:** [72604A](#)  
**Name:** **D-Luciferin, Na salt**  
**Formula:** CAS[103404-75-7], C<sub>11</sub>H<sub>7</sub>N<sub>2</sub>Na O<sub>3</sub>S<sub>3</sub>, M.W.= 302.30  
 White solid, water -soluble form of D-luciferin  
 Purity: >99%

**Storage:** Store at -20°C , desiccated and protected from light (L) (stable >6 months).  
 (All products) Long term storage at -70°C

Other products on inquire:  
 216390 DMNPE-caged luciferin (cross easily biological membranes)  
 M14160 6-amino-6-deoxyluciferin (ADL)

## Directions for Use

### Protocole 1: Luciferin Reporter Assay

Luciferin reagent preparation

-Dissolve 1 mM luciferin or luciferin salt, 3 mM ATP and 15 mM MgSO 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 18200 M<sup>-1</sup>Cm<sup>-1</sup>.

Luciferin Assay

1. Warm luciferin substrate reagent to room temp before starting.
2. Lyse cells using your cell lysis method.
3. Pipet 5-10 µl of cell lysate into a microplate. Use lysis reagent or buffer without cells as blank.
4. Prime luminometer with luciferin substrate solution according to manufacturer's instructions.
5. 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.

## Technical and Scientific Information

- D-Luciferin, a monomeric 61kDa protein, is a natural compound isolated from fireflies (*Photinus pyralis*) and other beetles and is a substrate for the enzyme luciferase. Our highly **purified synthetic luciferin** exhibits physical properties identical to those of natural luciferin. CQ testing ensures that it is the active form.
- ATP-dependent oxidation of luciferin by luciferase results in **bioluminescence** ( $E_m = 560 \text{ nm}$ ) at neutral and alkaline pH. Bioluminescence is red-shifted ( $E_m = 617 \text{ nm}$ ) under acidic conditions. This bioluminescent reaction is the most efficient known in nature, with about 90% of the energy released being converted to light.
- Because the **duration of the light output** is very brief, the luciferin substrate is injected into the samples by a luminometer instrument and the light output is measured for 5-10 seconds, with a 1-2 second delay because of its brief duration under usual assay conditions, and the light output that is proportional to luciferase concentration when substrates are present in excess (Mol Cell Biol 7, 725 (1987) ). Optimized formulations allows linear results over at >8 orders of magnitude of enzyme concentration, down less than 10–20 moles of luciferase. Generally, 50/100-fold greater sensitivity can be achieved over the chloramphenicol acetyltransferase (CAT) assay. A nearly constant signal for 1 minute or more can be achieved detected in single-tube luminometer or in a multiwell plate luminometer with an autoinjector.
- **Applications:** the luciferin/luciferase system is used as a very sensitive reporter assay for gene expression for plants, bacteria, mammalian cells, and for monitoring baculovirus gene expression in insects. It can too be used for ATP assays in research applications or to detect bacterial contamination. 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) ).
- In **gene reporter assays**, Luciferase is encoded by the luc gene as a 62000 product, which is widely used in a variety of cells. Because of the intrinsic low background of chemiluminescence technique, detection of the luc gene expression can be made at very low level. In addition, luciferin/luciferase has been used to measure  $10^{-15}$  molar quantity of ATP. Classically after incubation under the appropriate conditions, the cells are lysed and the cell extract is assayed for luciferase activity by incubation with the substrate luciferin, cofactor magnesium, and ATP. Luminescence can also be detected directly on cells, and even in-vivo.
- The free acid form is **soluble** in DMSO at pH >6, but may be dissolved directly in the Tricine or HEPES buffer used in the assay (at pH >6, max. ~1.5 mM). K and Na salts are soluble in water or aqueous buffer up to 100 mM at pH >6. Stock solutions of these salts can be made in ATP free water and stored frozen at -20°C. K and Na salts are easily used to formulate one own assay reagents for monitoring in vitro or in vivo luciferase activity.
- **Bioluminescence parameters:** Excitation occurs at 328nm, Maximum emission is at 532nm (free acid) or 533nm (Na, K salts); Molar extinction coefficient is  $18000 \text{ cm}^2 \text{ M}^{-1}$  (17000 for Na salt). Production of light can be monitored with either a luminometer or a scintillation counter.

### Literature Free acid Luciferin

"Amino Analogs of Firefly Luciferin and Biological Activity Thereof." White EH, et al. J Am Chem Soc 88, 2015 (1966) PN28743.

[PubMed](#): "Chemiluminescence microscopy as a tool in biomedical research." Creton R, Jaffe LF. Biotechniques 31, 1098-1100 (2001) PN45575.

[PubMed](#): [Article](#): "Visualization of ATP release in pancreatic acini in response to cholinergic stimulus. Use of fluorescent probes and confocal microscopy." Sorensen CE, Novak I. J Biol Chem 276, 32925-32932 (2001) PN45182.

[PubMed](#): [Article](#): "Catalytic activities of mitochondrial ATP synthase in patients with mitochondrial DNA T8993G mutation in the ATPase 6 gene encoding subunit a." Baracca A, Barogi S, Carelli V, Lenaz G, Solaini G. J Biol Chem 275, 4177-4182 (2000) PN36714.

[PubMed](#): "A convenient one-step extraction of cellular ATP using boiling water for the luciferin-luciferase assay of ATP." Yang NC, Ho WM, Chen YH, Hu ML. Anal Biochem 306, 323-327 (2002) PN47288.

[PubMed](#): "ATP luminescence-based motility-invasion assay." de la Monte SM, Lahousse SA, Carter J, Wands JR. Biotechniques 33, 98-100 (2002) PN47190.

[PubMed](#): [Article](#): "Low intracellular zinc impairs the translocation of activated NF-kappa B to the nuclei in human neuroblastoma IMR-32 cells." Mackenzie GG, Zago MP, Keen CL, Oteiza PI. J Biol Chem 277, 34610-7 (2002) PN47615.

[PubMed](#): [Article](#): "Functional interactions of lanthanum and phospholipase D with the abscisic acid signaling effectors VP1 and ABI1-1 in rice protoplasts." Gampala SS, Hagenbeek D, Rock CD. J Biol Chem 276, 9855-9860 (2001) PN44366.

### Literature Na salt Luciferin

Brasier, A. et al. (1989). Optimized use of the Firefly Luciferase assay as a reporter gene in mammalian cell lines. Biotechniques 7, 1116-1122. Lett. Appl. Microbiol. 1, 208(1990).

### Other Literature Luciferin

Gould, S.J. and Subramani, S. (1988). Firefly Luciferase as a tool in molecular and cell biology. Anal. Biochem. 175, 5-13.

Webster, J.J. and Leach, R.F. (1980). Optimization of the Firefly Luciferase Assay for ATP. J. Appl. Biochem 2, 1469-479.

DeLuca, M. and McElroy, W. (1984). Two kinetically distinguishable ATP sites in firefly luciferase. Biochem. Biophys. Res. Commun. 23, 764-770.

Bronstein, I. et al. (1994). Chemiluminescent and Bioluminescent reporter gene assays (Review). Anal. Biochem. 219, 169-181.

Alam, J. and Cook, J. (1990). Anal. Biochem. 188, 245-25.

DeLuca, M. et al. (1989). Firefly Luciferase: Mechanism of action, cloning and expression of the active enzyme. J. Biolum. Chemilum. 3, 1-5.

Rev.D03E