D(−)-Luciferin

D-(−)-2-(6´-Hydroxy-benzothiazolyl)-D_2-thiazoline-4-carboxylic acid
from *Photinus pyralis*, synthetic

Cat. No. 11 626 353 001 50 mg

Version 17
Content version: August 2018
Store in dark at −15 to −25°C

1. What this Product Does

Storage and Stability

Store the unopened reagent at −15 to −25°C until the expiration date printed on the label.

⚠ Store protected from light.

Luciferin is transferred to an excited state by light radiation and reacts then with air oxygen spontaneously.

⚠ The dissolved luciferin is stable for about 1 day at 0°C, if stored protected from light. Therefore, prepare the solution freshly each day. Do not freeze.

Application

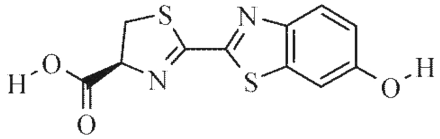
D(−)-Luciferin is the natural substrate of luciferase from firefly. It is used for the luminometric determination of Luc activity in cell extracts. Together with firefly luciferase it is used for the determination of ATP using bioluminescence.

Luciferase/luciferin may also be used to assay metabolites that can be converted to ATP (*e.g.,* AMP, ADP (1), cAMP (2) and enzymes that produce ATP (creatine kinase [3], myokinase [4]).

Luciferase/luciferin has also been used to measure the disappearance of ATP, as in luminometric assays of triglyceride (5), chloramphenicol (6) and aminoglycoside antibiotics (*e.g.,* gentamicin, kanamycin, neomycin) [7].

Product Characteristics

Molecular weight	280.3
Formula	$C_{11}H_8N_2O_3S_2$



Appearance	crystals
Purity	chromatographically homogeneous
Function test	performance-controlled in the luciferase assay

2. How to Use this Product

Working Concentrations

- For the assay of medium concentrations of ATP (10^{-9} – 10^{-6} M in the assay cuvette), use 35 – 70 μM D(−)-Luciferin (8).
- For the assay of low concentrations of ATP (10^{-13} - 10^{-8} M in the assay cuvette), use 350 μM D(−)-Luciferin (8).
- For the assay of metabolites convertible to ATP or enzymes which produce ATP, the literature suggests concentrations of D(−)-Luciferin from 35 – 359 μM.

Preparation of D(−)-Luciferin Solution

To minimize handling of the unstable compound, prepare a D(−)-Luciferin solution at the approximate concentration desired, then adjust it to the exact concentration on the basis of absorbance at 327 nm. (The absorptivity of D(−)-Luciferin at 327 nm is 18.2 $mmol^{-1} \times L \times cm^{-1}$).

For instance, to prepare a 700 μM solution of D(−)-Luciferin (8):

❶	Add 1.5 mg of D (−)-Luciferin to 5 ml of 70 mM Tris-acetate, pH 7.75 [theoretical concentration = 1.07 mM]
❷	Dilute a portion of that stock solution 20-fold with buffer.
❸	Read the absorbance at 327 nm.
❹	Add buffer to the stock so that a 20-fold dilution gives A_{327} of 0.637 (concentration of 20-fold dilution = 35 μM; concentration of stock = 700 μM).

Determination of ATP in the Concentration Range from 10^{-9} to 10^{-6} M (Final Concentration)

The following concentrations are optimal in the assay:

- assay buffer: 50 mM Tris acetate buffer, pH 7.75, 2 mM EDTA, 60 mM dithiothreitol*,
- bovine serum albumin*, 0.075% (w/v)
- 10 mM magnesium acetate
- **D(−)-Luciferin, 35 μM**

Luciferase* from *Photinus pyralis*, 500 – 5000 U/ml correspond to 0.05 - 0.5 mg enzyme protein/ml.

Before use, allow the vial to warm up to +15 to +25°C protected from light. Take about 1 mg substance (roughly weighed) and dissolve it in assay buffer at 0°C and protected from light (brown glass vial, ice-bath).

The exact adjustment of the concentration is carried out with the aid of the absorption coefficient $\epsilon_{327\,nm} = 1.82 \times 10_4$ [$l \times mol^{-1} \times cm^{-1}$ [Bowie, L. J. (1978) *Methods Enzymol.* 57, 15].

Determination of ATP in the Concentration Range from 10^{-13} to 10^{-8} M (Final Concentration)

The sensitivity of the measurement can be increased by using for example the Biolumat LB 9500, Fa. Berthold, Wildbad. For this assay the reagents – excluding the ATP sample – have to be mixed and pre-incubated for 12 – 24 h at +15 to +25° C.

** available from Roche Diagnostics*

3. Additional Information on this Product

3.1 Changes to Previous Version

-Editorial changes.

References

1 Hampp, R. (1985) in *Methods of Enzymatic Analysis*, Vol. 7, pp. 370-379.
2 Turner, G.A. & Mazlan, M. (1985) in *Methods of Enzymatic Analysis*, Vol 7, pp. 389-396.
3 Wulff, K. (1983) in *Methods of Enzymatic Analysis*, Vol. 1, pp. 340-368.
4 Brolin, S.E. (1983) in *Methods of Enzymatic Analysis*, Vol. 3, pp. 553-559.
5 Werner, M., Gabrielson, D.G. & Eastman, J. (1981) *Clin. Chem.* 27, 268-271.
6 Boeckx, R.L. & Brett, E.M. (1981) *Clin. Chem.* 27, 819-822
7 Daigneault, R., Larouche, A. & Thibault, G. (1979) *Clin. Chem.* 25, 1639-1643.
8 Wulff, K. & Doeppen, W. (1985) in *Methods of Enzymatic Analysis*, Vol. 7, pp. 357-364.

4. Supplementary Information

4.1 Conventions

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

4.2 Ordering Information

Product	Pack Size	Cat No.
Bovine Serum Albumin	20 mg (1 ml)	10 711 454 001
DTT	2 g	10 197 777 001
	10 g	10 708 984 001
	25 g	11 583 786 001
	50 g	11 583 786 001
	100 g	10 709 000 001

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