D-Luciferin Free acid, potassium salt, and sodium salt *UltraPure Grade*

Introduction

Luciferin is the most popular and versatile bioluminescent substrate. The firefly luciferase/luciferin bioluminescent system is found in the firefly (Photinus pyralis) and several other beetles. Luciferase oxidizes ATP-activated luciferin through a dioxetanone intermediate. Firefly luciferase produces light by the ATP-dependent oxidation of luciferin. The 560 nm chemiluminescence from this reaction peaks within seconds, with light output that is proportional to luciferase activity when luciferin and ATP are present in excess. Firefly luciferase has long been conjugated to antibodies and used as a label in immunoassays using luciferin as the substrate for detection. Compared to HRP and alkaline phosphatase, luciferase is less tolerant to chemical modifications. One particular advantage to the enzyme is that there is low endogenous luciferase activity in mammalian tissues besides its high sensitivity. Another important use of luciferase is in the area of hygiene monitoring. The luciferase/luciferin system can be used to detect contamination because ATP, present in all living organisms, is required to produce luminescence. The main application for this type of ATP bioluminescence is quality assurance by testing surfaces in food processing plants to determine whether or not there is contamination of either equipment or products.

Ordering Information

Cat. #	Product Name	Unit	MW	Ex (nm)	Em (nm)
12501	D-Luciferin, free acid *UltraPure Grade*	25 mg	280.32	328	533
12502	D-Luciferin, free acid *UltraPure Grade*	100 mg	280.32	328	533
12503	D-Luciferin, free acid *UltraPure Grade*	1g	280.32	328	533
12505	D-Luciferin, potassium salt *UltraPure Grade*	25 mg	318.41	328	533
12506	D-Luciferin, potassium salt *UltraPure Grade*	100 mg	318.41	328	533
12507	D-Luciferin, potassium salt *UltraPure Grade*	1g	318.41	328	533
12509	D-Luciferin, sodium salt *UltraPure Grade*	25 mg	302.3	328	533
12510	D-Luciferin, sodium salt *UltraPure Grade*	100 mg	302.3	328	533
12511	D-Luciferin, sodium salt *UltraPure Grade*	1g	302.3	328	533
12518	Amplite™ Luciferase Reporter Gene Assay Kit *Bright Glow*	1 plate	N/A§	328	533
12519	Amplite™ Luciferase Reporter Gene Assay Kit *Bright Glow*	10 plates	N/A§	328	533
12520	Amplite™ Luciferase Reporter Gene Assay Kit *Bright Glow*	100 plates	N/A§	328	533

§ The detailed protocols refer to the specific product insert

Storage Conditions

Store desiccated at -20 °C. Avoid light. Expiration date is one year from the date of receipt.

General Protocols

Note1: The D-luciferin salts are readily soluble in aqueous buffers 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.

Note2: The D-luciferin can be used with any existing reporter assay or ATP assay system.

Note 3: 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.

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.

1. Example protocol for *in vitro* bioluminescent image assays

1.1. 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.
1.2. Prepare a 0.5-1 mM working solution of D-Luciferin in pre-warmed tissue culture medium.
1.3. Aspirate media from cultured cells.
1.4. Add Luciferin working solution to cells, and incubate the cells for 5-10 minutes at 37 °C just prior to imaging.

2. Example protocol for *in vivo* bioluminescent image assays

2.1. Prepare a 15 mg/mL Luciferin stock solution in DPBS, w/o $Mg2^+$ and $Ca2^+$. Mix well.
2.2. 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.
2.3. Inject the luciferin intra-peritoneally (i.p.) 10-15 minutes before imaging at150 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.

3. Example protocol for luciferin reporter assays

3.1. 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.
3.2. Prepare a 1 mM working solution of D-Luciferin with 3 mM ATP, 1 mM DTT and 15 mM $MgSO_4$ in 25 mM tricine buffer pH 7.8.
3.3. Pipette 5-10 μl of cell lysate into a microplate. Use lysis reagent or buffer without lysate as a blank.
3.4. Prime luminometer with luciferin working solution according to manufacturer's instructions.
3.5. Inject 200 μl of luciferin working solution with no delay and a 10 second integration time.

References

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3. Viviani VR, Ohmiya Y. (2006) Bovine serum albumin displays luciferase-like activity in presence of luciferyl adenylate: insights on the origin of protoluciferase activity and bioluminescence colours. Luminescence, 21, 262.
4. Schipper ML, Patel MR, Gambhir SS. (2006) Evaluation of firefly luciferase bioluminescence mediated photodynamic toxicity in cancer cells. Mol Imaging Biol, 8, 218.
5. Oba Y, Sato M, Inouye S. (2006) Cloning and characterization of the homologous genes of firefly luciferase in the mealworm beetle, Tenebrio molitor. Insect Mol Biol, 15, 293.
6. Palomba S, Berovic N, Palmer RE. (2006) Bioluminescence of monolayers of firefly luciferase immobilized on graphite. Langmuir, 22, 5451.