

Product Information

D-Luciferin Potassium Salt

Catalog Numbers: DLP01, DLP02, DLP03

Description

D-Luciferin is a commonly used substrate for luciferase. D-Luciferin potassium salt is the potassium salt form of D-Luciferin and exhibits good water solubility. It can be directly dissolved in water or commonly used buffers without the need for alkaline dissolution, making it suitable for in vitro and in vivo bioluminescence imaging and related research applications.

In the presence of ATP and luciferase, D-luciferin undergoes an enzymatic oxidation reaction to produce bioluminescence. When D-Luciferin is present in excess, the bioluminescence intensity is positively correlated with the luciferase concentration within a certain range.

Plasmids carrying the luciferase-encoding gene (Luc) can be transfected into cells, which are then introduced into experimental animals such as mice or rats. Following administration of D-Luciferin, changes in the bioluminescence signal can be monitored using bioluminescence imaging (BLI), enabling real-time assessment of disease progression, drug efficacy, and other biological processes. In addition, changes in the bioluminescence signal can be used to detect and assess cellular ATP levels and energy metabolism.

Applications

- In Vitro Assays: Used for in vitro bioluminescence detection and quantitative determination of luciferase expression levels in biological samples.
- In Vivo Assays: Used for in vivo bioluminescence imaging (BLI) to monitor disease progression and assess drug efficacy in experimental animals in real time.
- Energy Metabolism Analysis: Changes in bioluminescence signals can be used to detect and assess cellular ATP levels and energy metabolism status.

Transportation and Storage

- Shipped at ambient temperature.
- Protect from light.
- Powder form: Stable for up to 3 years at -20°C.
- After reconstitution: Stable for up to 1 year at -80°C.

Instructions for Use

Upon receipt, promptly store the product under the specified storage conditions.

Depending on experimental requirements, prepare a stock solution of the desired concentration using an appropriate solvent (e.g., sterile water or sterile PBS). Aliquot the solution and store at -80°C. When needed, thaw at 4°C, allow the solution to equilibrate to room temperature, and dilute to the target concentration for addition into the experimental system. Avoid repeated freeze-thaw cycles.

The following experimental procedure is provided for reference only.

A. *In Vitro* Bioluminescence Imaging (BLI)

Reagents

- 1) D-Luciferin potassium salt
- 2) D-PBS
- 3) Cells of interest (HEK293T cells are used as an example in this protocol)
- 4) Plasmid (MA0509 is used as an example; concentration: 800 ng/μL)
- 5) Transfection reagent (Lipofectamine 2000)
- 6) DMEM cell culture medium (replace with the appropriate medium according to the cell type), trypsin, enzyme reaction buffer, etc.

Solution Preparation

- 1) 30 mg/mL D-Luciferin potassium stock solution: Accurately weigh 60 mg of D-Luciferin potassium salt solid, and fully dissolve it in 2 mL of D-PBS.
- 2) Working solution: Dilute the above D-Luciferin potassium stock solution with DMEM basal medium to prepare the working solution. The final concentration can be set at 300 µg/mL and adjusted according to experimental requirements.

Experimental Procedure

- 1) Cell culture: Seed HEK293T cells in a 10-cm culture dish with 10 mL cell suspension (approximately $5-8 \times 10^6$ cells per dish). Incubate overnight in a cell incubator at 37 °C with 5% CO₂ to allow cell attachment.
- 2) Cell transfection:
On the following day, replace the medium with 10 mL of serum-containing complete DMEM. Prepare the transfection reagent-DNA complexes following the instruction manual of Lipofectamine 2000, add the complexes to the culture dish, and continue incubation for 24 h at 37 °C, 5% CO₂.
- 3) Detection:
Harvest cells by trypsin digestion, dilute the cell suspension with DMEM basal medium, and transfer into a 96-well plate (n=3). Rapidly add an equal volume of the above working solution to each cell well using a multichannel pipette. Immediately measure bioluminescence signals with a microplate reader (one reading per minute for up to 20 min), or incubate at 37 °C for 5–10 min before BLI imaging.

B. *In Vivo* Bioluminescence Imaging

Materials

- D-Luciferin potassium salt
- D-PBS or PBS

Procedure

1) Storage and Working Solution Preparation

D-Luciferin potassium salt should be stored as a sealed solid at -20 °C in a dry environment and prepared fresh prior to use. For intraperitoneal (i.p.) injection, weigh an appropriate amount of compound and dissolve it in the solvent to prepare a 30 mg/mL stock solution. Once completely dissolved, the solution can be used directly for downstream experiments (filtration/sterilization is generally not required).

2) Kinetic Curve Acquisition and Imaging Frequency

Intraperitoneal or subcutaneous injection: Acquire images every 5–10 min and continuously monitor the bioluminescence signal for 40–60 min.

3) Imaging and Analysis

For imaging and quantitative analysis, images should be acquired during the stable signal plateau, when the bioluminescence signal is at or near its maximum and remains relatively stable.

Perform imaging analysis when the luminescent signal reaches a maximum and stable plateau phase.

Note: Signal emission is affected by administration route, animal species, body weight, and other factors. It is recommended to establish a luciferase kinetic curve for each animal to identify the optimal signal plateau and detection time point.

Notice

1. To protect operator safety and health, laboratory coats and disposable gloves must be worn during the entire experimental procedure.
2. Technical information supplied with this product is for customer reference, academic exchange, and scientific research only. Our company makes no warranty regarding the absolute accuracy or completeness of such information.
3. This product is for research use only. Use in clinical diagnosis, therapy, food, pharmaceuticals, or any human-related applications is strictly prohibited. Do not store this product in residential premises.

4. All animal procedures used in the method development and performance validation of this product strictly followed applicable laboratory animal ethics guidelines and regulations. Users conducting animal experiments shall comply with the animal ethics review and animal welfare requirements of their respective institutions.
5. Protect from light during storage and handling. Where feasible, the product solution may be purged with nitrogen or argon to reduce oxidation, enhancing product stability and shelf life.
6. Avoid repeated freeze-thaw cycles after dissolution. It is highly recommended to prepare a stock solution and store it in single-use aliquots.
7. **Important:** This product is non-sterile. If used for cell culture, appropriate sterilization or aseptic processing must be carried out prior to use.

Troubleshooting

Possible Problems and Solutions:

Symptoms	Possible Cause	Solution
The solution remains turbid after the product is dissolved in water	—	Dissolve the product using PBS buffer instead.
The bioluminescence signal decays too rapidly	The substrate undergoes fast metabolism and clearance <i>in vivo</i> .	Minimize imaging duration and capture images within the peak signal window .
Precipitation forms in the solution	The solution concentration may be excessively high, or the temperature may be too low.	Lower the concentration or incubate in a 37 °C water bath to facilitate dissolution.