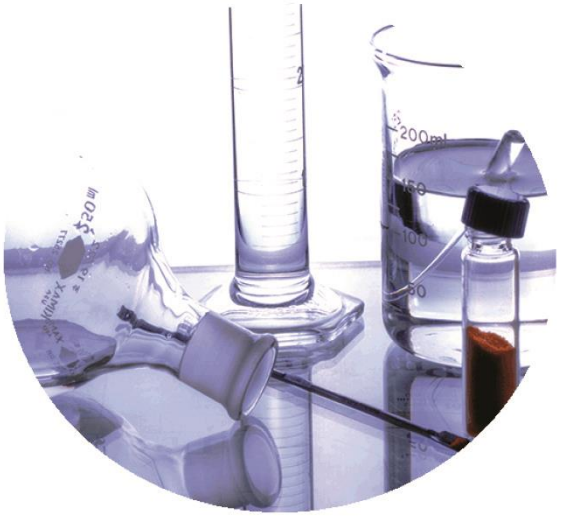

Transfection tools

# D-Luciferin, Sodium Salt

## Protocol

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The art of delivery systems

# IMPORTANT NOTES – Before you begin

✓ **D-Luciferin, Sodium Salt (Na+)** is a synthetic Firefly Luciferin.

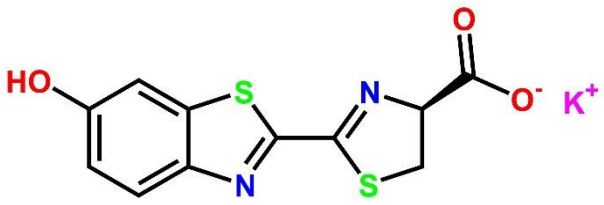
This highly pure and endotoxin-free product is perfect for in vitro and in vivo bioluminescent assays.

The highest quality of substrate eliminates possible interference in assays due to the presence of endotoxins. Proper packaging in amber vials and under neutral gas assures product integrity and stability.

This water-soluble substrate of Luciferase is ready and easy to use.

✓ **Main Features**

- High purity > 99%
- Good solubility and great sensitivity
- Reliable in vivo reporter for bioluminescent assays
- Endotoxin free (ideal for in vivo application)
- Suitable for in vitro experiments
- Easy to use
- Quick and easy distribution throughout the animal




✓ **D-Luciferin, Sodium Salt :**

- Chemical Name: 4,5-Dihydro-2-(6-hydroxy-2-benzothiazolyl)-4-thiazolecarboxylic acid sodium salt
- Molecular Formula: C11H7N2O3S2•Na.H2O
- Molecular weight: 320.32 g/mol
- CAS Number: 103404-75-7
- Molecular Structure: The molecular structures of Firefly and Beetle Luciferin are identical.
- Molecular biology grade and premium pure: >99% pure. Quality verified by nine independent criteria including HPLC and FTIR.

CAUTION: **D-Luciferin is sensitive to light, oxygen and moisture.**

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

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# D-Luciferin, Sodium Salt | Specifications

| | |
|---|---|
| Package content | LN10000<br>D-Luciferin Sodium salt (Na+) 1 g |
| Shipping conditions | D-Luciferin Sodium salt is shipped at room temperature |
| Storage conditions | Store D-Luciferin Sodium salt at -20°C or -80°C upon reception |
| Shelf life | 1 year from the date of purchase when properly stored and handled |
| Product description | D-Luciferin Sodium salt (Na+) is designed for use in in vitro and in vivo bioluminescent assays. The quality and purity of the D-Luciferin is essential to obtain good and reproducible results. |
| Important notice | For research use only. Not for use in diagnostic procedures |

# Applications

- Bioluminescent assays in living cells, tissues and animal models
- Luciferase reporter gene assays
- Whole animal imaging (in vivo reporter assay)
- Appropriate read-out for transfection/transduction with luciferase reporter gene and luciferase-fusion constructs
- ATP assays (Luciferase catalyzes conversion of ATP into AMP) and immunoassays
- Pyrosequencing
- Luciferase fragment complementation for sequential gene analysis experiments

# Protocols

The instructions given below represent successfully applied protocols. They can be used as guidelines to quickly achieve very high bioluminescence signal.

Optimal conditions do vary according to animals, cell cultures, route of administration and assay sensitivity.

D-Luciferin final quantity might have to be adjusted to achieve best results.

## 1. Preparation of D-Luciferin for in vivo assay

Biodistribution of the D-Luciferin is rapid and easy throughout the animal but kinetics may be tissue dependent. D-Luciferin can penetrate cell membranes and is able to pass blood-brain barrier, blood-placenta barrier and blood-testis barrier.

### Materials

- D-Luciferin, sodium salt (#LN10000)
- D-PBS (without Mg2+ and Ca2+)
- Syringe filter 0.2 µM

### Procedure

The best is to reconstitute the quantity of needed D-Luciferin for each experiment. However, stocks of frozen solution of Luciferin can also be used (see storage and stability section above).

1. Dissolve the D-Luciferin in D-PBS to obtain a final concentration of 15 or 30mg/mL.
2. Filter sterilize trough a 0.2µM filter (optional, not required).
3. Inject intraperitoneally or intravenously 5 to 15 minutes before imaging each animal with 10µL/g of body weight or 150 to 300 µg/g of body weight. To have a sufficient or even excess amount of substrate we recommend using 3mg of D-Luciferin per mice (100µL of 30mg/mL).

**Note 1**: we recommend performing **kinetic study** to determinate D-Luciferin kinetic curve and peak signal for your animal model:

i. Inject D-Luciferin as previously described. Awake or sedated animals can be used; however, with sedated animals the kinetics (peak luciferase expression time) may slightly be extended.
ii. After 3 min. if awaken animals were used in step 1, proceed to sedate animals.
iii. Place sedated animals in imaging chamber and capture the first image about 5 min. after the Luciferin injection.
iv. Continue to take images every 5-10 min. up to about 45-60 min. to produce a kinetic curve for Luciferin expression in your animal model.
v. Thereafter you can choose the best time point to image at. We image most of our models at 10-20 min. after D-Luciferin injection.

**Note 2**: for Intraperitoneal (I.P.) injection of D-Luciferin.

The animal needs to be in dorsal position (abdomen side up), manually controlled, with cranial end of animal pointed down. Injection has to be made in the animal's lower left abdominal quadrant. Needle (25 gauge) should be bevel-side up and slightly angled when entering the abdominal cavity. Penetrate just through abdominal wall (about 4-5 mm) and inject (1cc syringe).

## 2. Preparation of D-Luciferin for in vitro assay

### Material

- D-Luciferin, sodium salt (#LN10000)
- Molecular biology grade water
- Complete media

### Procedure

1. Prepare a D-Luciferin stock solution of 15 or 30mg/mL (100X or 200X) in molecular biology grade water
2. Mix by inverting the tube or bottle repeatedly until D-Luciferin is completely dissolved
3. At this step solution can be used immediately or aliquoted and frozen at -20°C or -80°C.
4. Add stock solution of D-Luciferin to pre-warmed tissue culture medium, the final D-Luciferin concentration should be 150µg/mL.
5. Remove old cell culture medium from the cultured cells
6.Add the D-Luciferin solution (150µg/mL) to cells immediately before imaging

**Note:** Cells can be incubated at 37°C for a short period of time before imaging to increase the signal

# NOTES

# NOTES

## Additional transfection tools products

- **DMNPE-caged luciferin**; a good alternative to D-Luciferin, used to measure luciferase activity in live cells.

- **X-Gal substrate** for staining transformed bacteria and LacZ transfected or infected cells, tissues and organisms

**Purchaser Notification**

**Limited License**
The purchase of the D-Luciferin kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the D-Luciferin kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all D-Luciferin kit reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a D-Luciferin kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the D-Luciferin kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

**Product Use Limitations**
D-Luciferin kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.


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Rev. 04/2021 FB