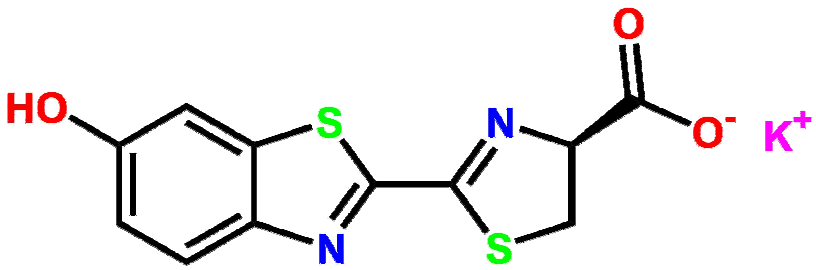
D-Luciferin Potassium Salt

INSTRUCTION MANUAL



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D-Luciferin, Potassium Salt

Instruction Manual

D-Luciferin Potassium salt (K^+) 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. OZ Biosciences offers high quality of **Endotoxin-Free** D-Luciferin K^+ salt

List of D-Luciferin products:

Catalog Number	Description	Weight
LK10000	D-Luciferin Potassium salt	1 g
LN10000	D-Luciferin Sodium salt	1 g

Use the table above to determine the appropriate catalogue number for your needs. You can order these products by contacting us. These products are also available in bigger quantities (2g, 5g, 10g etc.). Please contact us for a quotation at order@ozbiosciences.com. For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

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1. Description

1.1. Introduction and characteristics

Congratulations on your purchase of the **D-Luciferin, Potassium Salt** product!
D-Luciferin, Potassium Salt 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 are:

- High purity > 99.5%
- 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, Potassium Salt:

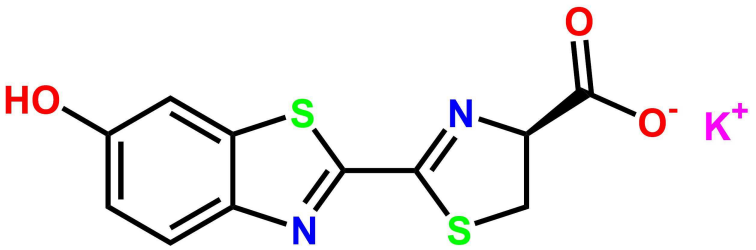
– *Chemical Name:* 4,5-Dihydro-2-(6-hydroxy-2-benzothiazolyl)-4-thiazolecarboxylic acid potassium salt

– *Molecular Formula:* $C_{11}H_7N_2O_3S_2 \cdot K$

– *Molecular weight:* 318.42 g/mol

– *CAS Number:* 115144-35-9

– *Molecular Structure:*



The molecular structures of Firefly and Beetle Luciferin are identical.

– *Molecular biology grade and premium pure:* 99.7% pure. Quality verified by nine independent criteria including HPLC and FTIR.

1.2. Kit content

D-Luciferin, potassium salt is providing in a 1.0g/vial in an amber bottle under nitrogen. D-Luciferin is sensitive to light, oxygen and moisture. This product is also available in larger quantities (2g, 5g, 10g etc.). Please contact us for a quotation at order@ozbiosciences.com

1.3. Stability and Storage

D-Luciferin is sensitive, to light, oxygen and moisture as powder and in solution.

Storage (powder) — Upon receipt and for long-term use, store the powder in a tightly closed and dessicated container, at -20°C. D-Luciferin is stable for at least one year at -20°C under desiccated and neutral gas conditions. If the bottle containing the D-Luciferin powder has to be opened and closed repetitively, it is recommended to fill the vial with an inert gas (Argon or Nitrogen) before closing.

Storage (solution) — D-Luciferin stock solution can be prepared in water or D-PBS and then frozen at -20°C or preferably at -80°C. Stability of the D-Luciferin solution is rather controversial and accordingly we recommend for the most sensitive studies (low concentrations of enzyme, sub-optimal temperatures or salt concentrations) to use freshly made solutions. Nonetheless, frozen D-Luciferin solutions (-80°C) have been suitable for use

after at least 6 months under neutral gas and adjusted pH conditions. D-Luciferin is quite unstable at low pH (< 6.5) and high pH (> 7.5).
To avoid repeated cycles of freezing and thawing, we recommend aliquoting D-Luciferin solution in small tubes and storing them at -80°C.

Shipping condition D-Luciferin is shipped at room temperature.

2. 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

3. Protocol

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.

3.1. 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, Potassium salt (#LK10000)
- 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).

3.2. Preparation of D-Luciferin for *in vitro* assay

Material

- D-Luciferin, potassium salt (#LK10000)
- 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

4. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)* / 3DfectIN *(for hydrogels culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g X-Gal powder 1g / G-418, Sulfate 1g

Purchaser Notification

Limited License

The purchase of the **D-Luciferin, potassium salt** product grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended **for internal research only** by the buyer. Such use is limited to the use 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.

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