# D-Luciferin (potassium salt)

## Introduction

D-Luciferin is a substrate of firefly luciferase. Firefly luciferase can catalyze the oxidative reaction of D-Luciferin in the presence of ATP, $Mg^{2+}$ and molecular oxygen, emitting a yellow-green light. Luciferin is widely utilized in biotechnology, particularly for in vivo imaging studies. It is used to track tumor cells, stem cells, or pathogens in animals like rats and mice. By injecting luciferin, researchers can monitor disease progression or treatment efficacy in real-time and non-invasively via Bioluminescence Imaging (BLI). Luciferin is also used for in vitro assays, such as luciferase and ATP assays, reporter assays, high-throughput sequencing, and contamination detection.

The three common forms of D-Luciferin are free acid, potassium salt, and sodium salt. The free acid form needs to be dissolved in alkaline solutions such as KOH or NaOH. The potassium and sodium forms are soluble in aqueous buffer. So, potassium and sodium forms are more suitable for various biological experiments, such as in vivo imaging analysis and in vitro bioluminescence detection.

## Components and Storage

| Components | C3654-50 mg | C3654-100 mg | C3654-500 mg | C3654-1 g |
|---|---|---|---|---|
| D-Luciferin (potassium salt) | 50 mg | 100 mg | 500 mg | 1 g |
| Store the reagent at -20°C away from moisture and light, stable for 2 years. | | | | |

## Protocol

### 1. In vitro bioluminescence assay

1) Prepare a 15 mg/mL D-Luciferin stock solution with $ddH_2O$. Use immediately, and store unused solution at -20°C in aliquots to avoid repeated freeze-thaw cycles.

2) Dilute the stock solution with pre-warmed culture medium to make a working solution (0.15-0.3 mg/mL). The concentration of the working solution can be adjusted according to the experiment.

3) When the cells have grown to the appropriate density, remove the culture medium.

4) Add D-Luciferin working solution to the cells and incubate in a 37°C cell culture incubator for 5-10 min prior to image analysis.

2. **In vivo imaging assay**

1) Prepare a 15 mg/mL D-Luciferin stock solution in sterile DPBS (without $Mg^{2+}$, $Ca^{2+}$) and mix well.

***Note:** Because calcium and magnesium ions may inhibit the activity of firefly luciferase, and magnesium ions may affect the oxidation of firefly luciferase, it is recommended to use DPBS or PBS without $Mg^{2+}$, $Ca^{2+}$.

2) Sterilize the luciferin solution with a 0.2 μm filter. Use immediately, and store unused solution at -20°C in aliquots to avoid repeated freeze-thaw cycles.

3) Inject the luciferin solution intraperitoneally (i.p.) with a dose of 150 mg/kg (or 10 μL/g of luciferin stock solution). Other injection methods such as tail vein injection or subcutaneous injection can also be selected according to the needs of the experiment.

4) Wait 10-15 minutes before imaging for maximum signal.

***Note:** It is recommended to perform a kinetic study for each animal model to determine the peak signal time.

3. **Firefly luciferase reporter assay (direct use of K2236 or K2246 or K1136 is recommended).**

1) Prepare a 50 mM of D-Luciferin stock solution with dd$H_2O$. Use immediately, and store unused solution at -20°C in aliquots to avoid repeated freeze-thaw cycles.

2) Prepare assay buffers (3 mM ATP, 1 mM DTT, 15 mM $MgSO_4$ in 25 mM Tricine buffer, pH 7.8) in advance. Dilute the stock solution of D-Luciferin in assay buffer to make a 1 mM working solution.

3) Add 200 μL of D-Luciferin working solution to 5-10 μL of cell lysate and mix well.

4) Perform detection without delay.

## Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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