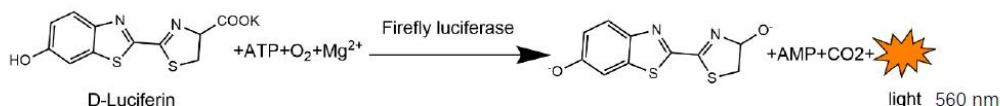


User Manual of CHASELECTION D-Luciferin Potassium Salt

Introduction:

D-luciferin is a commonly used substrate for luciferase, which reacts with luciferase in the presence of Mg^{2+} and ATP to form a dioxetane structure and produce luminescence. Under the condition of excessive fluorescein, the number of photons produced is positively correlated with the concentration of the enzyme. This type of luminescent reaction can be used to initiate and produce luminescent phenomena. Under the condition of excessive fluorescein, the number of photons produced is positively correlated with the concentration of the enzyme. This type of luminescence reaction can be used for experiments such as promoter optimization, drug screening, and cell ATP level analysis.



D-luciferin potassium salt has good water solubility and lipid solubility, and can be better applied in research such as in vitro and in vivo bioluminescence imaging compared to D-fluorescein (free acid).

Product information

Cat No.	Name	Package
CY057F0100	D-luciferin potassium salt	0.1g
CY057F1000	D-luciferin potassium salt	1g

Main Application:

Reporter gene analysis

In vitro bioluminescence analysis of cells

In vivo imaging analysis

High sensitivity ATP analysis

Research on other luciferases and their genes

Storage and Validity:

The product is shipped with blue ice. Store in a dark and dry place at -20°C , with a validity period of 1 year.

The working fluid is prepared and used immediately. Store the storage solution in sub packages at -20°C to avoid repeated freezing and thawing, and use it within 3 months.

Properties:

Alias	D-D-fluorescein potassium salt; 5-Fluorofluorescein
CAS	115144-35-9
Molecular formula	$C_{11}H_7N_2KO_3S_2$
Molecular weight	318.4
Appearance	Yellow powder
Solubility	Soluble in water
Purity (HPLC)	$\geq 99\%$

Product instructions:

1. In vitro Imaging

1) Preparation of storage solution

Dissolve 1.0g of D-luciferin potassium salt in 33.3mL sterile water to prepare a storage solution of 30mg/mL, or prepare the D-luciferin potassium salt solution required for a single experiment.

2) In vitro Bioluminescence Assay of Living Cells

- a. Inoculate cells overexpressing firefly luciferase on a culture plate;
- b. Dilute the prepared D-luciferin potassium salt storage solution with cell culture medium to 150 $\mu\text{g/ml}$ working fluid
- c. Remove the culture medium from the cell culture plate and add D-luciferin potassium salt working solution to incubate at 37 ° C for 10 minutes before using it for cell imaging.

2. In vivo imaging

1) Preparation of D-luciferin potassium salt solution

Dissolve 1.0g of D-luciferin potassium salt in DPBS (w/o Ca^{2+} , Mg^{2+}) to prepare 15mg/mL, or prepare the required D-fluorescein potassium salt solution for a single experiment, using 0.2 μM filter membrane filtration for sterilization.

2) In vivo Bioluminescence detection

- a. Determine the injection volume by 10 $\mu\text{L/g}$ (body weight). Each mouse receives 150 mg of D-luciferin per kg (body weight) (e.g. for mice weighing 10 g, 100 μL are required to provide 1.5 mg of D-luciferin potassium salt)
- b. Inject D-luciferin potassium salt solution into the abdominal cavity 10-15 minutes before in vivo imaging, or determine the specific time based on the kinetic curve.

3. Kinetic curve of luciferase activity in model animals

- 1) Dissolve D-luciferin potassium salt at 15mg/ml in DPBS (w/o Ca^{2+} , Mg^{2+}) and inject intraperitoneally at a working dose of 150mg/kg
- 2) Wait for 3 minutes, then give the animal routine anesthesia (both gas anesthesia and needle anesthesia are acceptable)
- 3) Place the anesthetized animal in the imaging room and take the first image.
- 4) Take continuous photos every 5-10 minutes for 40 minutes to obtain the absorption kinetics curve of D-luciferin potassium salt in the model animal.
- 5) Determine the optimal imaging time based on the dynamic curve.

Note:

- 1) The optimal imaging time is influenced by injection methods, animal types, and body weight. Therefore, it is recommended to perform a luciferase kinetic curve for each experiment to determine the optimal signal platform period and detection time.
- 2) If ATP related testing is required, try to avoid contamination from exogenous ATP as much as possible. If gloves are worn and ATP free experimental consumables are used during operation, ATP free sterile water should be used for the dissolution of fluorescein.
- 3) This product needs to be protected from light and stored. After filtration and sterilization, the storage solution can be packaged and frozen at -20 °C or -80 °C. If conditions permit, the storage liquid can be filled with nitrogen or argon gas (to prevent oxidation).
- 4) When dissolving D-luciferin potassium salt, DPBS without calcium and magnesium ions should be used. Calcium and magnesium ions may inhibit the activity of luciferase, and magnesium ions may affect the oxidation of fluorescein, thereby affecting detection.
- 5) This product is for research use only!