ATTO

CL-S100
CL-S1000

CLuc Substrate Instruction manual

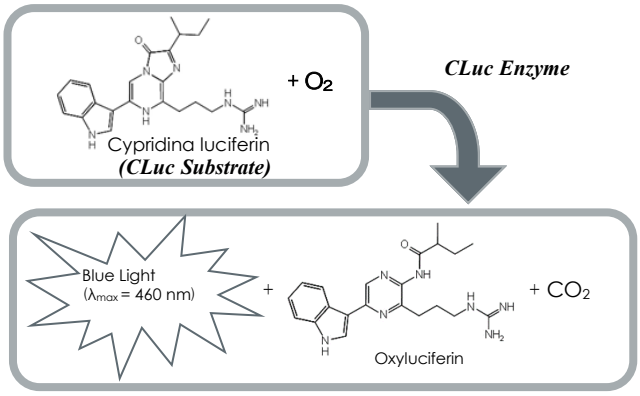
Rev. 6

1. For safe use

In order to use this product safely, please read this instruction manual carefully first. Please refrain from operation until you fully understand the contents of this instruction manual. In addition, this instruction manual describes only how to use this product for specified purposes. Please refrain from using for purposes and methods not specified in this instruction manual. In the unlikely event that the product is used for any purpose or method not specified in this instruction manual, the person who operates it is responsible for all necessary safety measures and unexpected situations.

2. Purpose of use

This product is a luciferin that is a substrate of Cypridina luciferase (CLuc) derived from *Cypridina noctiluca*. It emits blue light when mixed with CLuc.



3. Product composition

Name	Volume	
CLuc Substrate	100 reactions	1000 reactions
CLuc Solution A (for dissolution and preservation)	1 mL	1mL
CLuc Solution B (for assay)	10 mL	100 mL

4. Storage

> CLuc Substrate should be stored at -80 °C for both dry and dissolved enzyme solution. The dried product and the enzyme solution after dissolution can be stored for 6 months.

> Store CLuc Solution A and CLuc Solution B at 4 °C. The expiration date is 6 months (unopened).

5. Discard

> After use, please comply with the disposal rules of your institution.

> CLuc Substrate (Brown bottle)

Cap; Polypropylene
Bottle; Silicate glass
Inner lid; Butyl rubber

> CLuc Solution A (Screw cap tube)

Cap/Tube; Polypropylene
O-ring; Ethylene / Polyethylene

> CLuc Solution B (Black bottle)

Cap; Polypropylene

6. Prepare

> Ethanol

> Sample solution containing CLuc

> Luminometer

For multi-well plate
AB-2350 Phelios (ATTO)

For test tube
AB-2270 Luminescencer Octa (ATTO)

7. How to use

STEP [1] Dissolution / stock of CLuc Substrate

1. Add 1 mL of ethanol to 1 mL of CLuc Solution A to make a total of 2 mL, and mix **gently.**
2. Add CLuc Solution A (ethanol added) to CLuc Substrate bottle and mix gently.

   CLuc Solution A (etanol added) addition volume
   CL—S100; 110 µL
   CL-S1000; 1100 µL

   *It also dissolves the substrate on the bottle wall and rubber cap.
3. Cover the bottle and incubate at 4 °C for 10 minutes to dissolve the substrate.
4. Invert the bottle again to mix the substrate, and leave it at 4 °C for 15 minutes to completely dissolve CLuc Substrate.
5. Dispense completely dissolved CLuc Substrate into microcentrifuge tubes and store at -80 °C.

   *Freezing and thawing of the substrate will cause a decrease in bioluminescence intensity.

STEP [2] Measurement

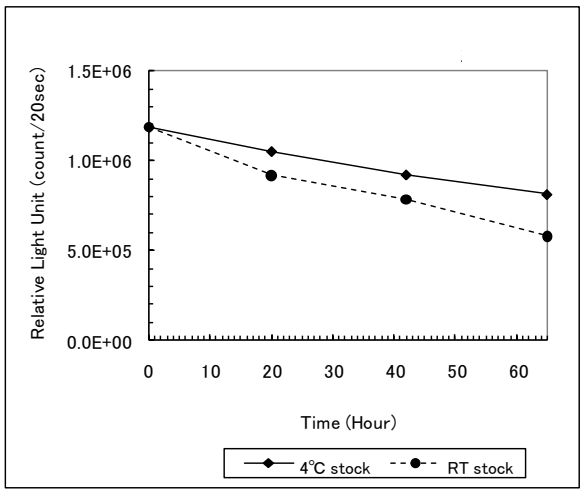
1. Dilute CLuc Substrate stock solution completely dissolved with CLuc Solution B to 75-fold.
2. 75 µL of diluted CLuc Substrate working solution and 25 µL of CLuc containing sample solution are mixed at room temperature.

3. Immediately (within 30 seconds) the lbiouminescence is measured with a luminometer.

* Dilute CLuc Substrate stock solution with CLuc Solution B immediately before measurement. Use the diluted CLuc Substrate working solution in the shortest time possible (Fig. 1).

* The bioluminescence reaction of CLuc is stable (Fig. 2). Stable data can be obtained by starting the measurement after the reaction with the sample reaches the plateau (after about 1 second).

* If the bioluminescence value of the sample is too high, dilute it with an appropriate buffer (medium, etc.).

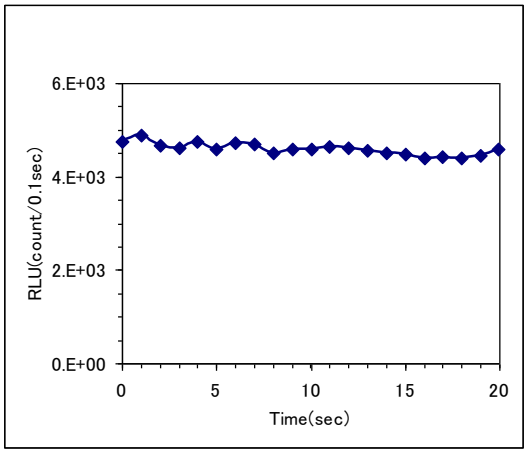


[Fig.1] Stability of diluted CLuc substrate solution

25 µL of the CLuc enzyme solution and 75 µL of the diluted CLuc Substrate solution were mixed, and the bioluminescence was measured with a luminometer (ATTO Luminescencer).

> When stored at 4 °C, the bioluminescence decreases by about 10% after 24 hours.

> When stored at room temperature, the luminescence value decreases by about 20 to 30% after 24 hours.



[Fig. 2] CLuc bioluminescence kinetics

25 µL of the CLuc enzyme solution and 75 µL of the diluted CLuc substrate solution were mixed, and the bioluminescence kinetics was measured with a luminometer (ATTO Luminescencer). The CLuc bioluminescence reaction is stable for at least 20 seconds.

8. Note

*** CLuc assay in yeast**

CLuc stability decreases under acidic conditions. Generally, the yeast culture medium shifts to the acidic side by culture, so it is recommended to add a final concentration of 0.2 M potassium phosphate buffer (pH 6.0) to the medium in advance in order to suppress CLuc inactivation.

For more information, refer to the "Tipps for Yeast CLuc Reporter Assay" document.

9. References

1) Nakajima, Y., Kobayashi, K., Yamagishi, K., Enomoto, T., and Ohmiya, Y. (2004) cDNA Cloning and Characterization of a Secreted Luciferase from the Luminous Japanese Ostracod, *Cypridina noctiluca*. *Biosci. Biotechnol. Biochem.*, **68(3)**, 565-570

2) Yamagishi, K., Enomoto, T., and Ohmiya, Y. (2006) Perfusion-culture-based secreted bioluminescence reporter assay in living cells. *Anal. Biochem.*, **354(1)**, 15-21

3) Wu, C., Suzuki-Ogoh, C. and Ohmiya, Y. (2007)Dual-reporter assay using two secreted luciferase genes. *BioTechniques.*, **42**, 290-292

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