4 Absorption measurement of Fluorescence

4.1 Preparation of coelenterazine solution

4.1.1 Preparation of stock solution (1mM)

Centrifuge the coelenterazine for a short time or oscillate vigorously, then place it at room temperature for 20 min, gently open the lid (in case of the powder spattering), add 2.5 mL anhydrous ethanol, cover the lid, centrifuge or oscillate for 10 s, and divide the solution into 1.5 mL centrifuge tubes, 100 ul for each tube, 25 tubes, and store at -20 ℃ away from light.

4.1.2 Preparation of working solution (5 μM)

Add 900ul ddH_2O (100 μM) to 100 μL stock (1mM), and then add 10 μL diluted stock to 190 μL bacterial fluid or algal fluid (96 well plate) to obtain the working concentration of 5 μM.

Attention：

1) To guarantee the Coelenterazine activity，limit the time for the lid to exposure to the air, and the whole process is wrapped with tin foil paper to reduce contact with external light.
2) If the coelenterazine is stored for a short time (several hours), it should be placed in a refrigerator at 4 °C and at -20 °C for a long time. Before use, resolve it at room temperature and confirm that there is no precipitation when taken out from the refrigerator. If there is sediment, it will be resolve in a water bath at 50 °C.

4.2 Preparation of Crude enzyme

4.2.1 Experiments

High Speed Tabletop Refrigerated Centrifuge (Eppendof 5804R), Ultrasonic Cell Disruption System(SCIENTZ-IID).

4.2.2 Material

Cell breaking buffer:10 mM KH_2PO_4 , 10 mM K_2HPO_4 , 500 mM NaCl, 20 mM imidazole，pH 7.4, 0.45 μm filter membrane.

4.2.3 Procedures

1) Collect fermentation broth in batches and centrifuge it in 50 mL centrifuge tube at

10000 rpm for 10 min.

2) Weigh 2~ 2.5 g of *E. coli* in 50 mL centrifuge tube, add 1 g bacteria in 7.5-8 mL of cell fragmentation buffer, and vortex until fully resuspended cells.
3) Under the condition of power 300 W and time interval 1:2 (s/s), to lysis the *E. coli* by ultrasound sonication in ice water bath for 20 min.
4) Centrifugate bacteria cells at 4 ℃,10000 rpm for 20 min and collect the supernatant. Resuspend the precipitation with 3 mL buffer, then centrifuge it. And mix the supernatant of 2 times centrifugation. Store the supernatant at -20 ℃ for protein concentration determination and SDS-PAGE gel electrophoresis.

4.3 The measurement of fluorescence / chemiluminescence

Equipment： Fluorescence spectrophotometer (Hitachi F-4500)

4.3.1 The measurement of fluorescence

After the 3 mL of an appropriate fluorescent substrate were introduced into a quartz cuvette. the luminescence intensity emission was measure using a changeable excitation wavelength and fixed emission wavelength. If the detection exceeds the maximum limitation, it should be diluted properly.

4.3.2 The measurement of fluorescence chemiluminescence

After mixed up appropriate crude enzyme solution and bacterial solution or algae solution, adding 100 μL of coelenterazine working solution, and measure chemiluminescence immediately after incubation for 10 min at room temperature in the dark. Turn off the excitation wavelength, scan the emission wavelength and emission wavelengths of 480 nm were selected.