# Specification

| AB-2350 PHELIOS　Code No.3511140 | |
|---|---|
| Sample format | 96/384 well plate format |
| Detector | Photomultiplier（PMT）<br>Photon counting methods |
| Spectral range | 350～670 nm |
| Filters | F0: no filter, F1: 560nm LP, F2: 600nm LP |
| Color separation | Up to three luminescence colors can be separated with automatic filter change mechanism |
| Injectors | Built-in plunger type<br>96 well:　20 to 250 uL<br>384 well: 20 to 100 uL<br>Additional pump system (optional, available for only 96 well plate) |
| Temperature control | Ambient＋5 ℃～40℃ |
| Counting times: | 96 well 1 to 1200 sec, 384 well 1 to 600 sec |
| Data saving | 200 files of measurement results, 9 files of calibration curve<br>Exporting and data saving to PC through Windows interface program |
| Size | 398 (W) × 460 (D) × 286 (H) mm/16.5 kg |
| Power | 100-240V 50/60Hz 100VA |
| Standard set: | PHELIOS (Built in one pump), Control Software (Windows), USB cable, AC cable, Manual |
| **Options** | |
| Code No. 3511150 | AB-2020M PHELIOS (Phelios equipped with dual pump system) |
| Code No. 3511068 | Single-side and double-side fitting tube |

AB-2270 Luminescenser OCTA

AB-2280 Luminescencer Octa-NIR

AB-2550 KronosDio

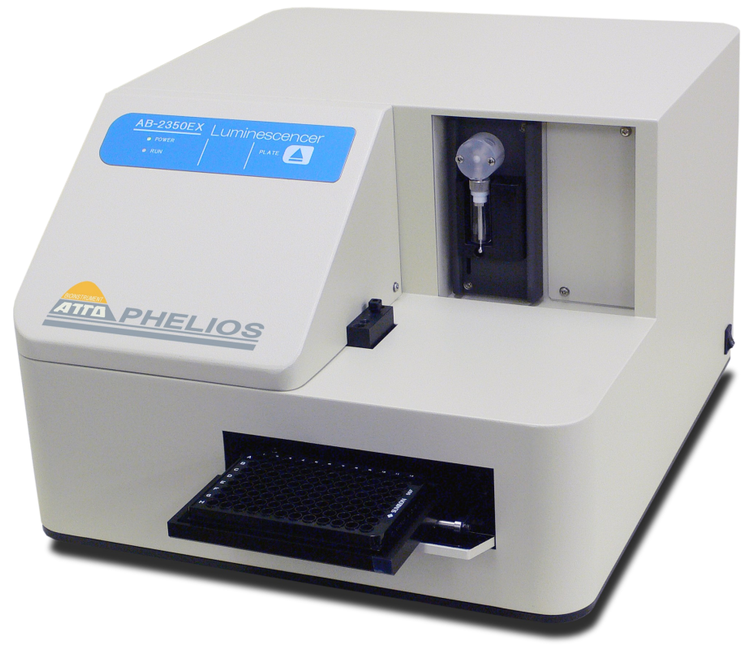

**ATTO Corporation**

3-2-2 Moto-asakusa, Taito-ku, Tokyo 111-0041, JAPAN

Tel: +81-3-5827-4863　Fax: +81-3-5827-6647

Luminometer for 96/384 well plate

# AB-2350 PHELIOS



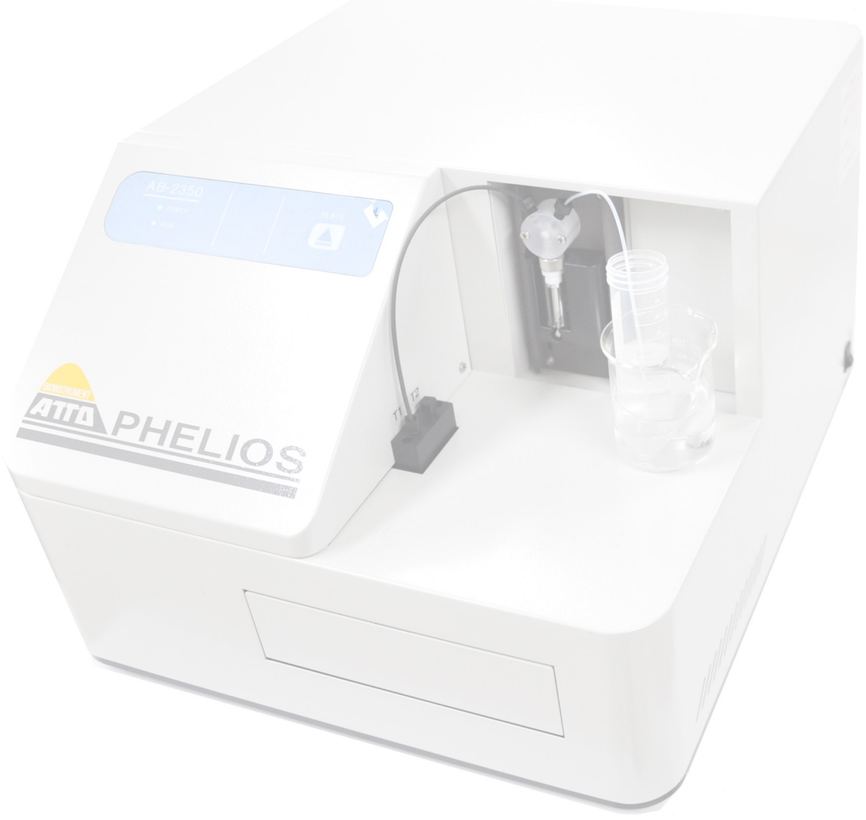

Made in Japan　Bioscience & Biotechnology

The development history for ATTO's luminometer was started from 1980 with luminescent detector for HPLC. Since then we have been developed measurement methods for biological activities of living cells and tissues by bioluminescence and chemiluminescence, and finally we commercialized microtiter plate reader "Luminescencer" for automatic luminescence measurement in 1991.

Afterwords, ATTO released "Luminescencer JNR" as a luminometer for microtiter plate in 1997, and followed by releasing "Luminescencer PSN" as a luminometer for microtubes. Thus we have been consistently producing for various product range as a specialized manufacturer for luminescence .

Furthermore, ATTO has made efforts to develop basic technologies such as bioluminescent imaging system, active oxygen measurement system, multi-color separation system, luminescent spectrophotometer, novel secretory luciferase (Cluc), and also studied the mechanism of firefly luciferase reaction, global standardization of luminescent measurement, and near infrared measurement technology.

"ATTO Phelios" offers superior performance for measuring bioluminescence and chemiluminescence in flash and glow assays with high sensitivity and reliability, and it is useful tool for diverse applications from ELISA to cell based assays.



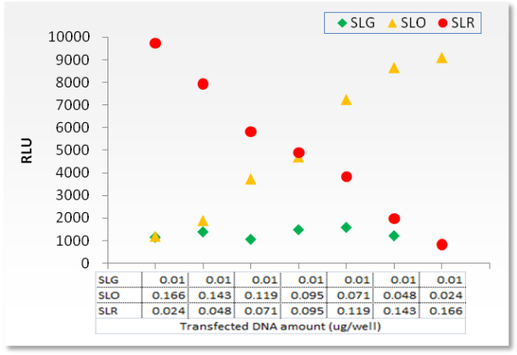

# Application Data

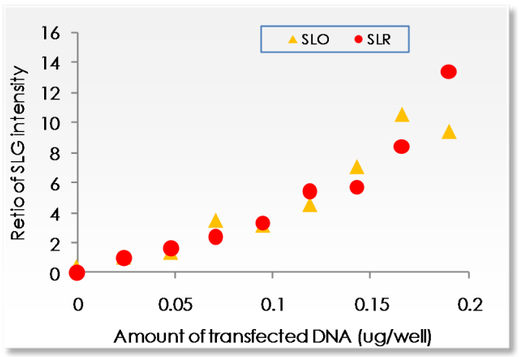
## Triple Reporter Assay

The result shows the bio-luminescent intensities of tri-color luciferases in NIH3T3 cells measured by using Phelios. The bioluminescent intensities are in proportion to the amounts of transfected DNA, since Phelios enables to measure and analyze individual colors of luciferases in single cell.

[Method]

NIH3T3 cells were transfected the control vectors (Tripluc) , pSLG-SV40 control, pSLO-SV40 control and pSLR-SV40 control (purchased from TOYOBO, JAPAN) with lipofection method. Next day, the medium is changed to the fresh one including 0.2mM of luciferin, and measured accumulated bioluminescence intensities for 10sec. using F0, F1 and F2 filters with Phelios.



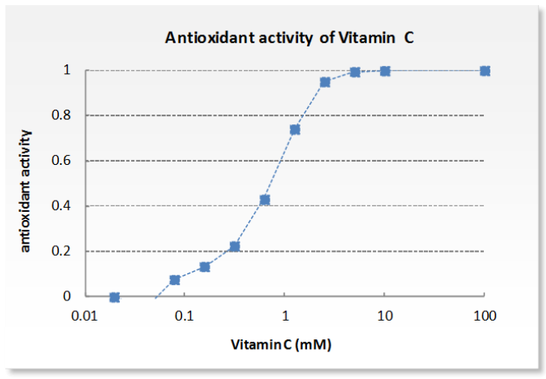



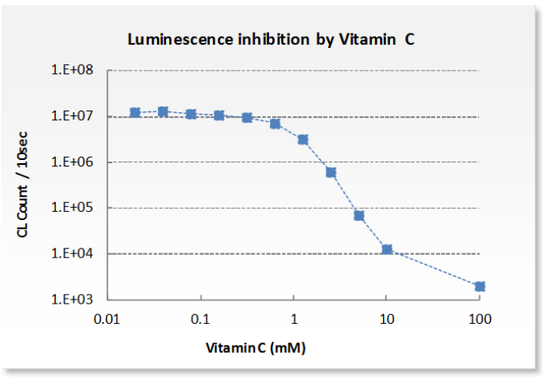

## Measurement of Anti-oxidant activity

Anti-oxidant activities of food ingredients (substances) can be compared using bio-luminescent agents (such as MCLA, MPEC, NIR-CLA,,,) that reacts against ROS(Reactive Oxygen Species). Anti-oxidant activities can culculate from the ratio of bioluminescent intensities between samples with and without ROS inducer. Generally, the anti-oxidant activity is evaluated by the concentration of anti-oxidant substance which is reached to 50% of maximum bio-luminescent intensity, and this concentration is commonly compared with the concentration of water soluble vitamin E.

[Method]

The measurement of anti-oxidant activities in vitamin samples were carried out using Phelios (AB-2970) and CRETA-S. The concentration of vitamin C was prepared from 40μM to 100mM, and the concentration of the vitamin C which is reached to 50% of the relative luminescent intensity was examined. As a result, the bio-luminescent intensity was reached to 50% when the concentration of vitamin C was about 1mM.



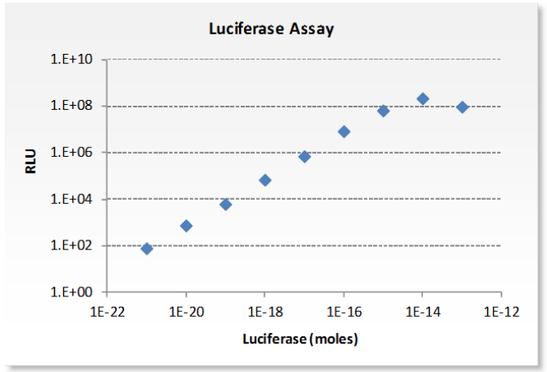
# Application Data

## Luciferase Assay

Phelios enables to detect at least 1zmoles($10^{-21}$ moles) of luciferase molecules. Phelios can be useful for quantitative measurement of widely concentrated sample in linearity dynamic rage (log 7).

[Method]

Set the 96 well black plate onto the plate holder of Phelios that added 20μL of luciferase diluted solution (Triplet) in each well. It measured bioluminescence accumulated from 2sec and 10sec after auto injection with 80μL of luciferin by injec-

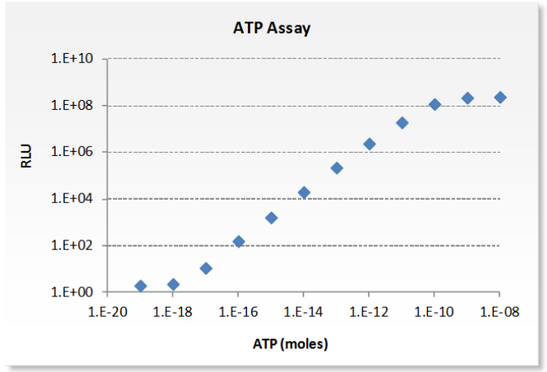



## ATP Assay

Phelios enables to detect up to 1 amoles ($10^{-18}$ moles) of ATP molecules, over more linearity dynamic range shows quantitative measurement in wide concentration samples up to log 8.

[Method]

Ten mL of ATP diluted solution was added in each well of a 96 well black plate, and set it onto the plate holder of Phelios. It measured accumulated bioluminescence intensity from 2sec to 10sec after auto injection with 90μL of luciferase-substrate reagents for ATP detection.

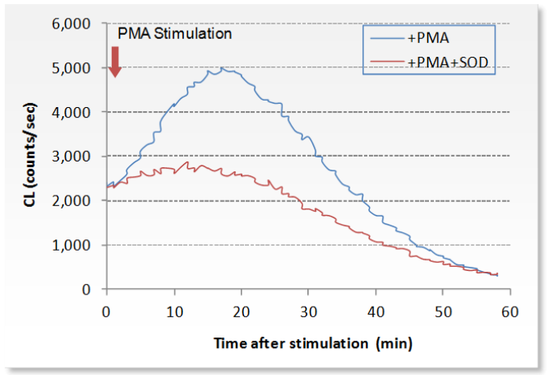



## Autophagy

The studies related autophagy such as oxidative stress of mitochondria and PKC signal pathway has been progressing. It is available to observe intracellular changes in the amounts of active oxygen generation for long time (~1h) by Phelios.

[Method]

HL60 cells (1.8 x $10^5$) were added 100 ng of PMA, and the changes of generation amounts of active oxygen was detected with MCLA (Bioluminescent reagent). It reveals that active oxygen was generated by PMA stimulation, since relative bioluminescence intensity should decrease by SOD addition.

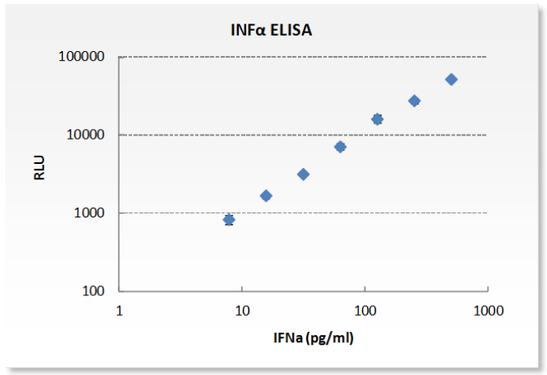


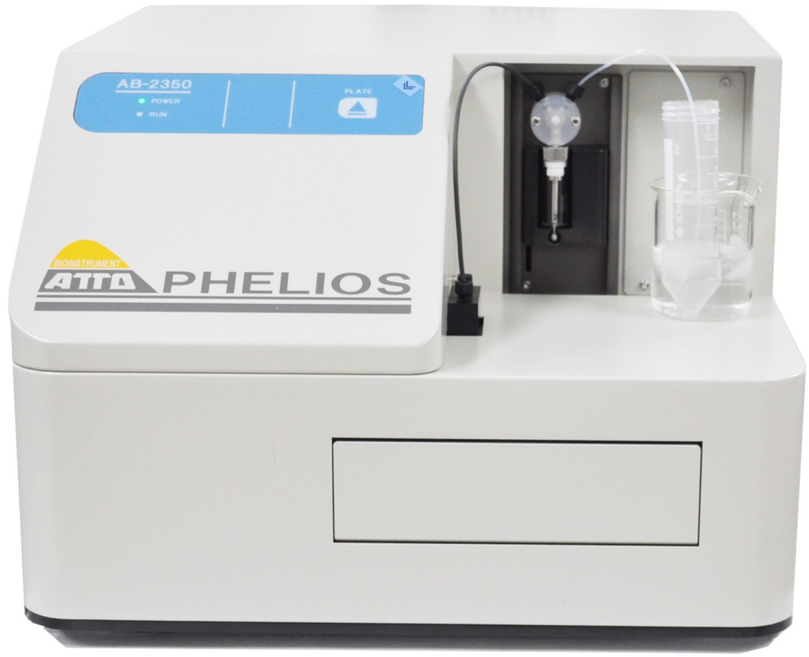

## High-sensitivity Immunoassay

Interferon alpha was detected by sandwich immunoassay method (CLEIA) with Biotin-labeled anti-INF alpha antibody and Cypridina luciferase labeled streptavidine. The result showed at least 10pg/mL of INF alpha could be detected with Phelios.

[Reference]

C. Wu, K. Kawasaki, Y. Ogawa, Y. Yoshida, S. Ohgiya, Y. Ohmiya : Anal. Chem., 79, 1634 (2007).




## Introduction

AB-2350 Phelios is a microplate luminometer developed to intend to measure multi samples with the highest efficiency of light detection. Phelios is available to measure diverse wavelength of bioluminescence from 350 to 670nm in 96, 384 well microplate. It achieves highly sensitive detection with added and extremely desired optical system for efficiency of luminescence by the technology of low noise photon counting. Besides, it accomplishes high reproducible measurement by internal temperature controller and highly accurate pump in repeated injection. Moreover, even in case of different multi-colors luminescent generation, it is available to measure the samples respectively by dedicated automatic filtering function.

## Features

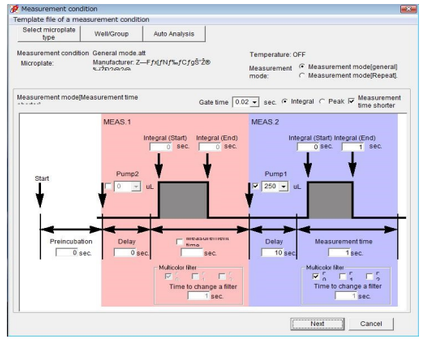
- Wide wavelength range detection between 350-670 nm
  →Red luminescence can be detected with high sensitivity.
- Wide dynamic range (8 logs)
- High sensitivity
  →Detection limit is 1zmole ($10^{-21}$ moles) of luciferase molecules.
- Temperature control system (Ambient+5℃~40℃)
  →Offer appropriate condition to measure light from living cells and tissue samples.
- Auto injection system
  →Programmable injector condition which enable to detect flash-based luminescence
- Color separation system
  →Tricolor of luminescence can be separated and analyzed automatically with filter system (560LP, 600LP)
- Measuring samples from flash-based light (20 msec) to long period observation (7 days)

# Simple Operation

## 1. Set appropriate reagent to injector

Prime injector line with appropriate reagents. Reagent volume for rinse and prime of injector line is only 340µL.

## 2. Input the measurement condition

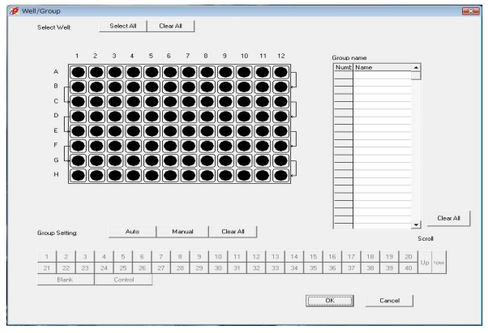



Select plate type (96/384 well plate) and set the measurement condition, such as injection volume, measurement time, delay time, filter and so on. Dual injectors (optional) are available, which allows to automatic analysis of dual reporter assay with two kinds of substrate reagents.

## 3. Set test plate on the plate holder

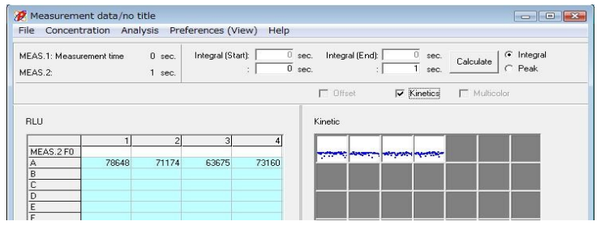

Push "PLATE" button to open and set the plate on the holder. Then push "PLATE" button again to close.

## 4. Start measurement

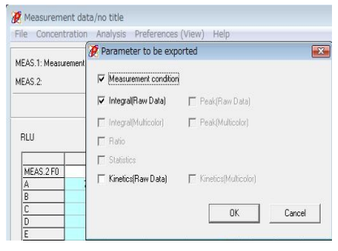



Select the measurement wells and click "OK" to start measurement.

## 5. Analyze the measurement results




Measurement results of individual luminescent intensities are plotted at the interval of 20 msec

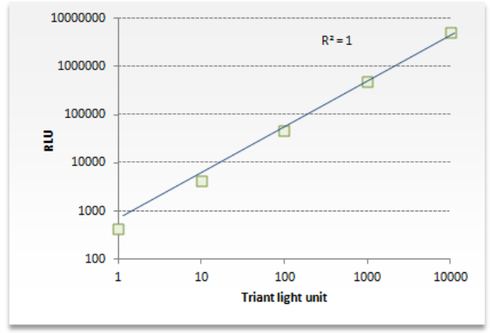



Analysis functions are available to make calibration curve, calculation for individual values of dual reporter assay. Measurement results and analyzed data can be exported as an "Excel" format.

# Basic Specification

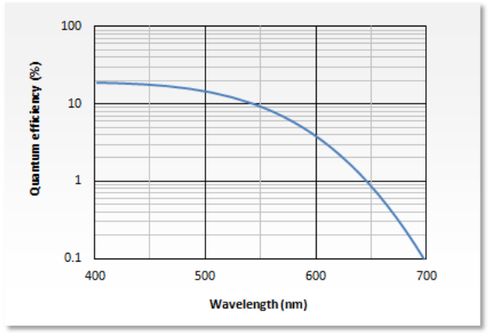
## Linearity

Light intensities of green LED as a standard light source were measured for 5 seconds. As a result, stable linearity was shown in the range from 1 to 10000 light units (light quantity of TRIANT ).



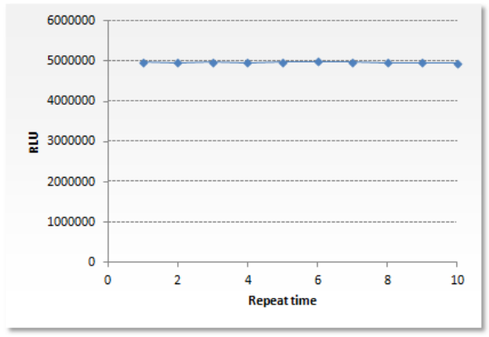

## PMT sensitivity

The PMT (photomultiplier tube) equipped in Phelios is adapted through severe inspection test for the lowest noise and high efficiency in long wavelength. ATTO also has the device for near infrared detection. Please feel free to ask us.



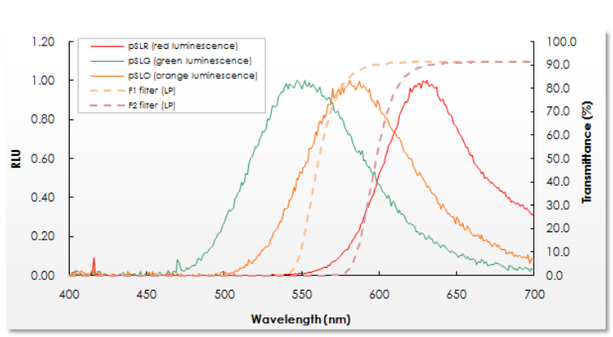

## Reproducibility

The reproducibility of Phelios was confirmed by repeated measuring the light intensity of green LED of standard light source TRIANT for 5seconds. The result showed high reproducibility with low CV value of around 0.21%. The background value was around 10 counts/sec.




## Automatic multi-color filtering system

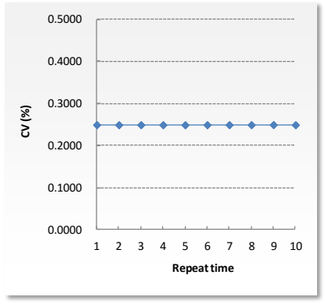
Phelios installed automatic multi-color filtering system which can distinguish up to 3 different colors luciferases by two different filters. By this technology and Tripluc (TOYOBO), it accomplishes analysis of three different genes expression with one substrate.




## Injector pump

The injector pump equipped in Phelios is high accurate for repeated injection and optimizing flow rate. The pump demonstrated CV values of 0.05% in 10 times of 250µL injection test, and 0.5% in 10 times of 30µL injection test. It reveals high accuracy in each injection test. To confirm the imperative injection speed, the reaction of Aequorin and Ca2+ ion which is usually in second was analyzed with Phelios. After injection of Ca2+ ion with injector pump, bioluminescence of Aequorin generated immediately and reached the maximum light intensity, and then it eventually deminished gradually. The pump is also suitable for high performance even for flash bioluminescence.

**Reproducibility of injector**




**Flash by Aequorin reaction**