Luminometer for 35mm Dish with CO_2 incubator system

AB-2550 Kronos Dio

Since 1964

ATTO

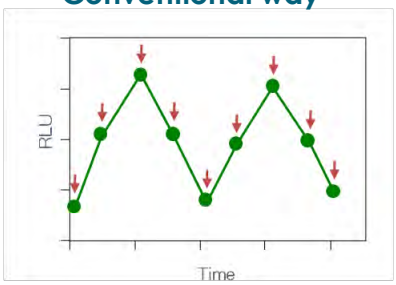
Made in Tokyo

SupportingLifeResearch

Bioluminescence monitoring in long term

Kronos Dio (AB-2550) is a luminometer for detection of bioluminescence in living cells and tissues by PMT. It is available to control temperature and CO_2 gas concentration inside Kronos Dio incubator suitable for cell & tissue culturing. Besides Kronos Dio gives superior performance for multi-color assay, which can detect different colors of luminescence up to three in the same time by internal optical filtering system. Kronos Dio possesses high sensitivity for detection very dim and weak luminescence consecutively in culture samples such as cells and tissues in 35mm culture dishes for a long time. Therefore, it is not required to prepare several samples for measuring each time point to chase alteration in chronologic bioluminescence. In this way, it is very reasonable and convenient against conventional way and method in terms of saving time, accuracy and efficiency.

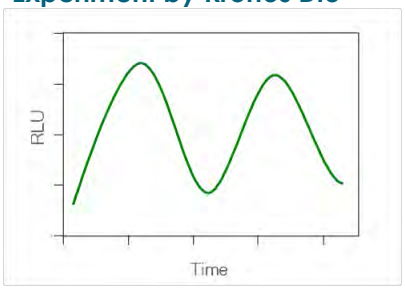
Conventional way



Lysis & measurement
Lysis & measurement
Lysis & measurement
Lysis & measurement
Lysis & measurement
Lysis & measurement
Lysis & measurement
Lysis & measurement

Necessary for sampling at each time-point

Experiment by Kronos Dio



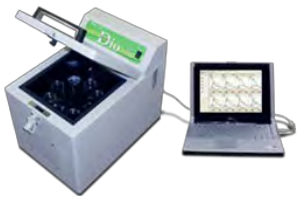
Consecutive measuring

Unnecessary for sampling at each time-point

Easy & Speedy !

Save your samples & reagents !

High efficiency & accuracy !



Compactly designed body of Kronos Dio contributes to save a working space (28(W) x 40(D) x 33(H) cm) . It is suitable for culturing cells and tissues under the environmental control such as temperature, CO_2 gas and humidity inside Kronos Dio incubator.

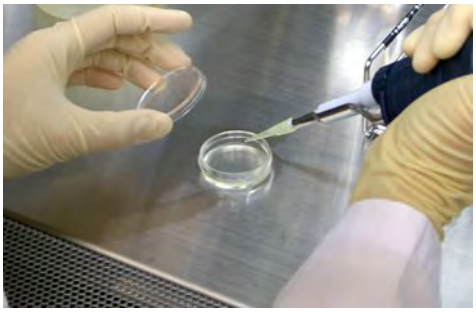
It is available to detach the sample dish table for 8 pcs of 35mm culture dishes from the Kronos Dio incubator so as to move and set samples very easily. Needless of negative control because it can use the luminescent value measured during shutter closing for subtraction of background. Furthermore, it can calculate up to 3 different color luminous intensity in the same time with inputted color separation coefficient(*) from the same sample emitting different color luminescences by internal optical filtering system .

*Ref: The color separation coefficient means transmittance values of F1/F0 and F2/F0 of each of colors in bioluminescence.

Measurement of Bioluminescence up to three colors

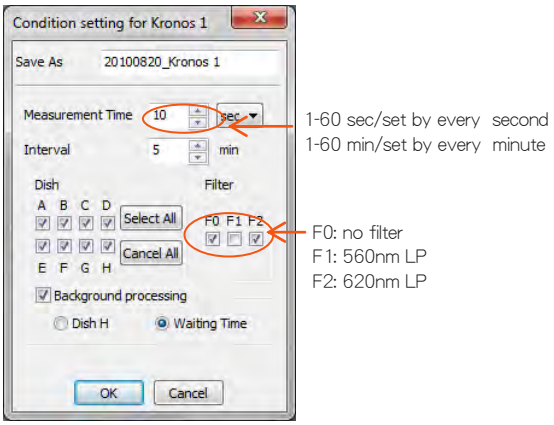
- Real-time Monitoring of luminescence from cells and tissues in 35mm culture dishes
- Adjustable environmental conditions as temperature, CO_2 gas and humidity
- Incredibly high specification of PMT for detection of dim and weak biolumi-nescence
- Measurement for 3 different colors of bioluminescence in the same time
- Complete control by PC with dedicated software
- Benefit to save working space by compactly designed body

Simple methods for luminescent measuring



1. Addition of luminescent reagents

Add luminescent reagents as luciferin to samples. Necessary to use the reagents that penetrate inside cells.

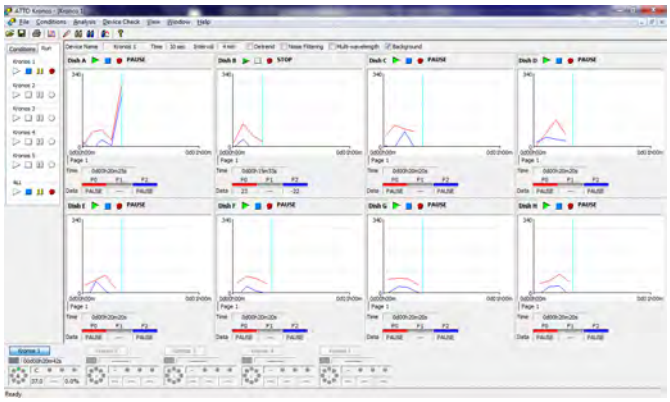


2. Condition setting

Select measurement time & filters. Three lumines-cent color can be measured in the same time.

3. Set samples

Set φ35mm culture dishes on the sample dish table up to 8 dishes.

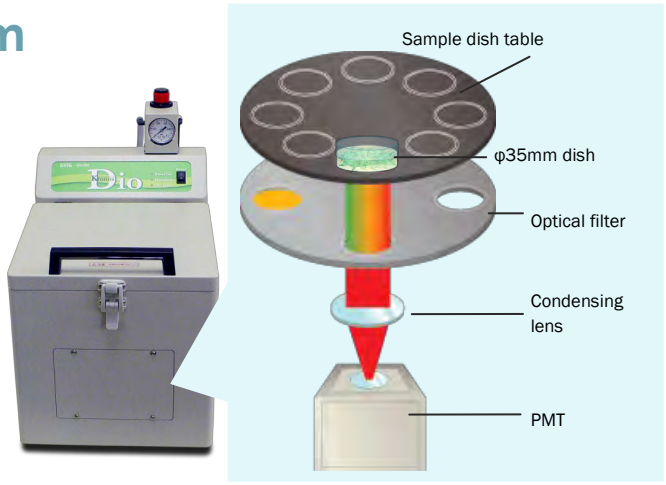


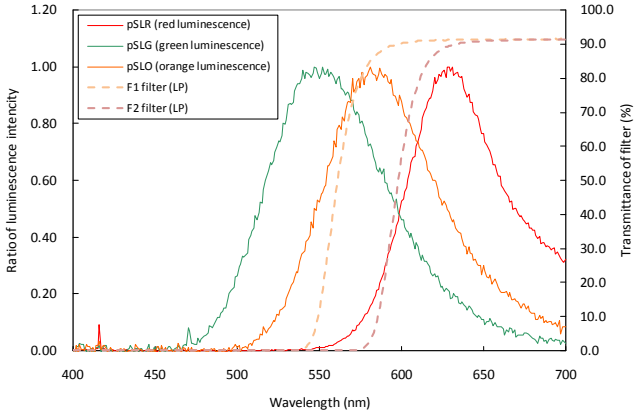
4. Start measuring

Click "Start button" ▶ to start measuring.

Multi-color separation system

The inside structure of Kronos Dio incubator is shown as the right diagram. Kronos Dio is equipped the round sample dish table carrying up to 8 culture dishes, which is designed to avoid cross talk between each of dishes. The optical filtering system is located under the sample dish table and inverse PMT is placed in the lowest position under the condensing lens located under filtering system. The automatic optical filtering system is achievable for multi-color separation. The luminescence transmitted through each filter is concentrating with condensing lens, and is measured by PMT.





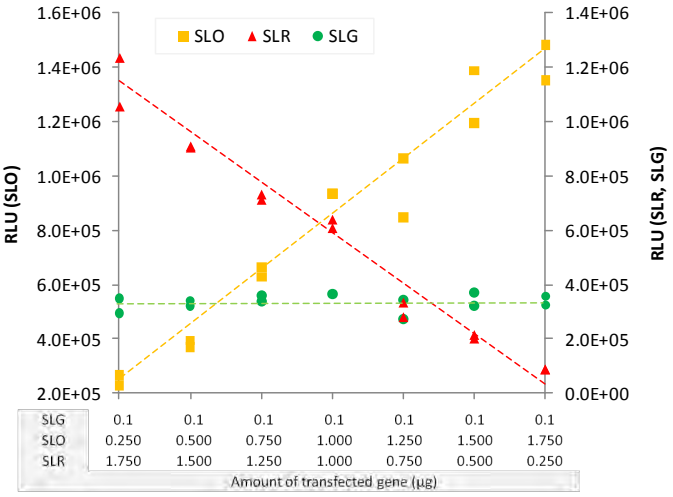
ATTO-original color separation method (Patent No. 4052389)

Two features of the ATTO-original color separation measurement method are 1) full light is measured, and 2) fewer filters are used for color separation than in conventional methods. As a result, filter-caused loss in luminescence is minimized, and actual value-comparable data can be obtained. The software of Kronos Dio can automatically calculate luminescence value for each of colors by inputting of each filter transmittance ratio into multi-color separation function window. (* Filter transmittance ration is a value of F1/F0 and F2/F0 against each of colors in bioluminescence)

The left graph is shown the result measured by Kronos Dio bioluminescences in NIH3T3 cells expressing 3 different color luciferases. Kronos Dio can measure bioluminescence in living cell chronologically and analyze each color luminous intensity of luciferases in the same time from the same samples expressing three color luciferase genes. The result is demonstrated that luminous intensity values can be obtained according to the amount of transfected DNA.

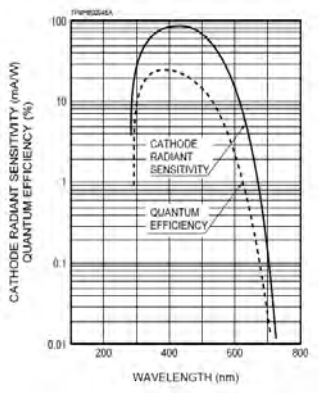
[Experimental protocol]

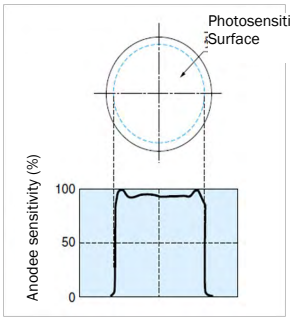
NIH3T3 cells seeded on 35mm culture dishes were transfected with each amounts of DNA such as pSLG-SV40 control vector, pSLO-SV40 control vector, and pSLR-SV40 control vector (Tripluc control vector, from TOYOBO) by lipofection. One day after transfection, the media is replacement to fresh media including 0.2mM luciferin, and the luminescence of samples were measured by Kronos Dio for 1 min of exposure time with F0, F1, and F2 filters.



Highly sensitivity & high accuracy

A detectable range of PMT determined by material of window that retains in vacuum, and meterial of photocathode that converts struck light into electron. In bioluminescence since it is visuable range of luminescence it usually determined by meterial of phtocathode. In using of the device equipped PMT, it differs obtained amount of signal from different wavelengh of luminescence even in the same amount of light. Because it depends on the wavelengh of quantum efficiency in photocathode. Since Kronos dio using PMT of wide range, it is available to detect effectively even red color luminescence range in long wavelength. Besides, by using flat type of PMT against photocathode, it can avoid effects that possibly occurred from location of sample or ununiformity of seeding cell, and carry out experiments with high accuracy.



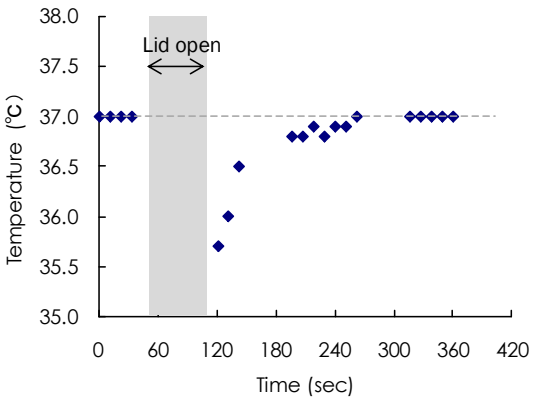


from Hamamatsu photonics catalogue

Optimized environment for cell and tissue culturing

Kronos Dio equips optimized environmental container for long-term observation with living cells and tissues by providing proper CO_2 gas concentration and temperature stably. Since it introduced system adjustable constant temperature by peltier, it can set 20-45ºC when the room temperature is at 25ºC, and can retain them constantly for a long time. The right graph shows recovery efficiency of temperature inside Kronos Dio incubator. The temperature test was carried out that the lid of Kronos Dio is opened for one minute, and then closed. The temperature alteration was monitoring during these process. The result was shown that it takes two minutes to recover the temperature to the original set point, and the temperature is reached stable status three minutes after closing lid. Thus, it can be applied with additional process such as addition of reagent or replacement of sample during measurement under minimizing the effect of environmental condition.

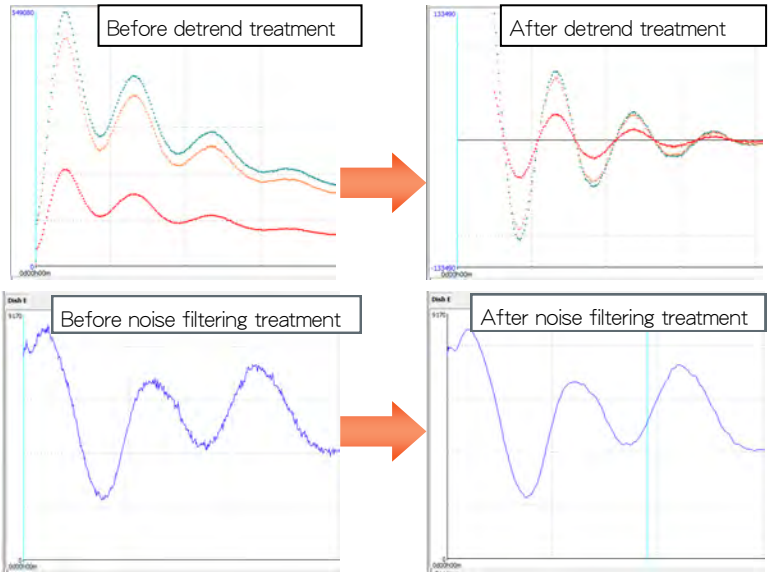
Temperature recovery inside incubator



When using tissue sections as your sample...
Set tissue sections on the culture insert (such as

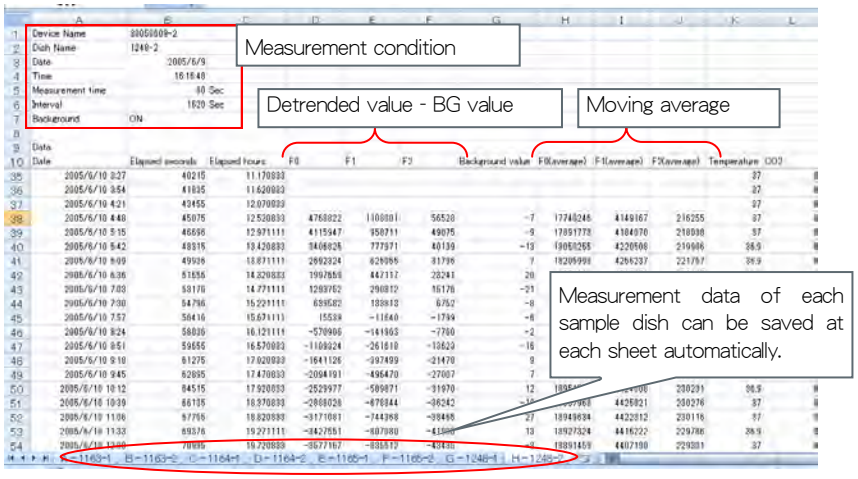
Data processing

- **Detrend**
  To facilitate making conspicuous the periodic fluctuation in expression of an observation target.
- **Noise filtering**
  To smooth the measured data.
  Two methods are available for the noise filtering: “Moving average” and “Median filter.”
- **Multi-wavelength**
  Measurement by separating color components of multi-color luminescence.
- **Background**
  To enable background subtraction.



Export & Save data

Measured data can be saved manually during measurement or after measurement in the format compatible with Microsoft Excel. A single file containing the data sheets for all the sample dishes that are selected will be created automatically. All of the measured data over the measurement time, temperature data, comments that are entered, etc. will be included. All of the graphs for a selected sample dish are saved collectively in the BMP/JPEG/TIFF format.



Applications of Kronos Dio

- **Real time reporter gene assay**
  - Transcriptional factor activity
  - Clock gene (chronopharmacology)
  - Gene expression (Transfection, RNAi,,,),,,
- **Analysis of cell responses**
  - Drug response (Anti-cancer drug, DDS,,,)
  - Stress response (Hormone, Inflammation, Anti-Oxidant)
  - Cytotoxichology,,,
- **Analysis of signal pathway**
  - Signal cascade (Calcium)
  - Apoptosis,,,

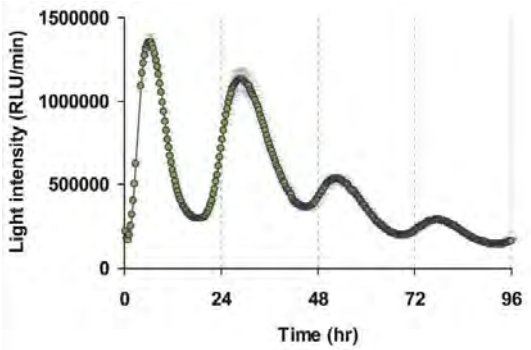
	Bioluminescence	Fluorescence
Probes	Luciferase, Aequorin,,,,others	GFP, YFP, RFP,,,,others
	Enzyme substrate (luciferin,,,etc.) Unnecessary external light	Excitation light Unnecessary substrates
Advantages	Low background Quantitative analysis No-damage to cells Long-term analysis	High resolution High intensity
Disadvantages	Low intensity	High background Photo-bleaching Damage to cells

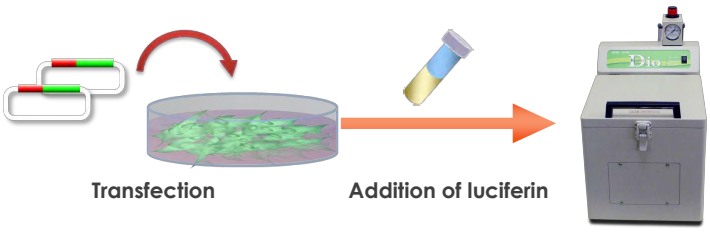
Quantitative analysis
Long term dynamics

Intracellular localization
Short term dynamics

Real-time Reporter Assay of Live-cell

Bioluminescence reporter assays are used in various studies of biological functions, such as gene expression, signal transduction, post-translational modification, protein interaction,,,etc. Our improved technologies of detection system for Luciferase assays have achieved the quantitative visualization of gene expression and monitoring of very weak luminescence signals in live-cell for long-term.
NIH3T3 cells expressed Bmal1 promoter fused to Luciferase gene as a reporter gene was stimulated synchronization of circadian rhythm by adding dexamethasone. Monitoring of luminescence in cultured NIH3T3 cells with Kronos-Dio were started by adding of 200 μM D-luciferin potassium salt. Measurement time: 60 sec, Experiment time: 4 days

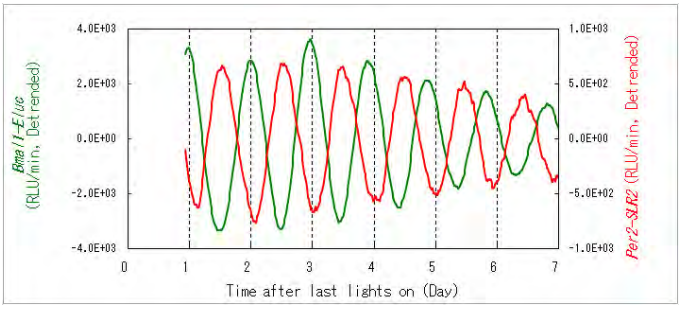


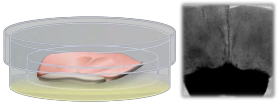


Data Supported: Dr. Y. Nakajima, AIST, JAPAN

Monitoring of multiple circadian gene expression

Multiple luciferase gene expression can be separated by differences in individual luciferase luminescence color and monitored them simultaneously for long-term with Kronos Dio.
Dual color luciferase assay of brain tissue section from transgenic mice expressing circadian gene promoter fused to luciferase, Bmal1-driven ELuc (Green luminescence) and Per2-driven SLR2 (Red luminescence) is shown. The bioluminescence of Bmal1-driven ELuc (cpm) is plotted with green lines and shown on the left y axis. The bioluminescence of Per2-driven SLR2(cpm) is plotted with red lines and shown on the right y axis. The x axis shows the time after last lights-on for the animals. The expressions of Bmal1 and Per2 in tissue section were stable for long-term and showed antiphase. Graph data were smoothed and detrended.





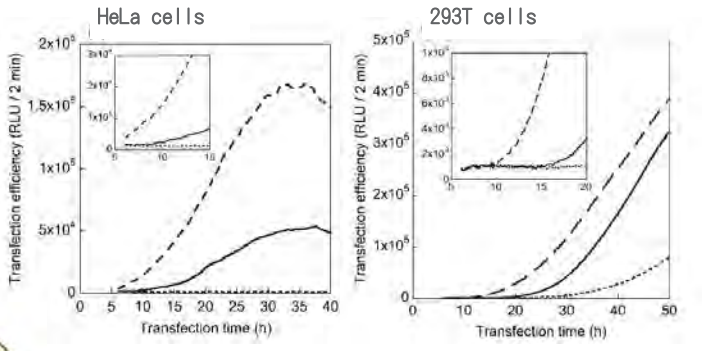
Brain tissue section in culture insert

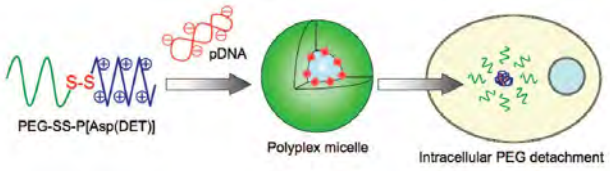
Multiple gene expression

Data Supported: Dr. Y. Nakajima and Dr. Y. Ohmiya, AIST, JAPAN
Reference: T. Noguchi et al. Biochemistly, Vol.49 (2010)

Time-dependent profiles of transfection efficiency

Time-dependent profiles of transfection efficiency with novel DDS carriers against HeLa and 293T cells.
The cells were incubated with each polyplex in DMEM containing 10% FBS for 6 h, followed by incubation in DMEM containing 10% FBS and 100 μM D-luciferin in the absence of polyplexes. The time shown in the x-axis started from the addition of polyplex solutions and the measurement satarted from 6 h. The inserts are expanded figures from 5 to 15 h.

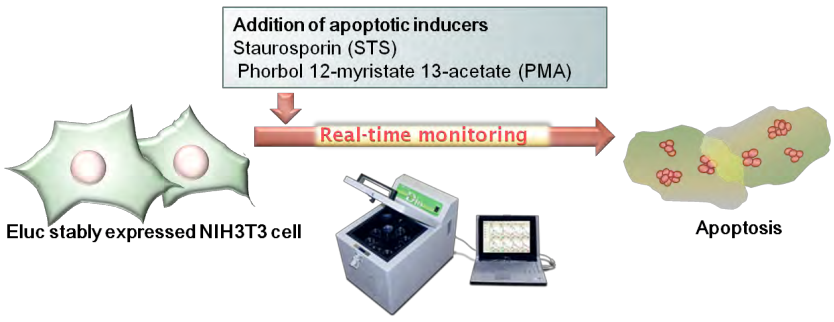


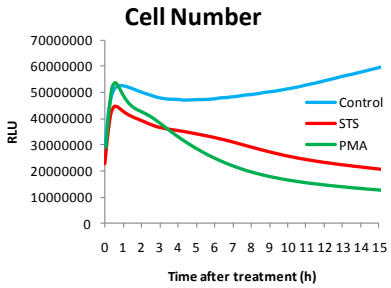


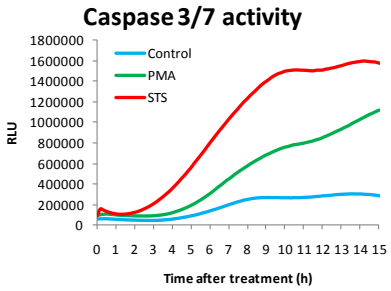
Data Supported: Dr. K. Itaka, Tokyo univ., JAPAN
Reference: S. Takae et al., J.Am.Chem.Soc., 130 (2008)

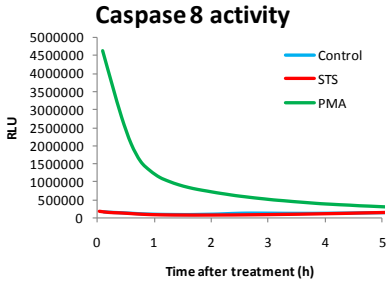
Analysis of Apoptosis

Kronos Dio can examine apoptotic events continuously by using luminescence probes. Luciferase gene stably expressing NIH3T3 cells were treated with apoptosis inducing agents, Staurosporin (STS) or Phorbol 12-myristate 13-acetate (PMA). After adding 0.2mM Luciferin, Caspase-Glo® 3/7 substrate (Promega) or Caspase-Glo® 8 substrate (Promega) to the medium, luminescence was measured with Kronos Dio for 1 min of exposure time at interval of 10 min.









References

1. F. Sato, M. Kurokawa, N. Yamauchi and M. Hattori, Gene silencing of myostatin in differentiation of chicken embryonic myoblasts by small interfering RNA. *Am. J. Physiol., Cell physiol.*, **291(3)**, C538-C545 (2006)
2. J. Hirayama, S. Sahar, B. Grimaldi, T. Tamaru, K. Takamatsu, Y. Nakahata and P. Sassone-Corsi, CLOCK-mediated acetylation of BMAL1 controls circadian function. *Nature*, **450**, 1086-1090 (2007)
3. S. Takae, K. Miyata, M. Oba, T. Ishii, N. Nishiyama, K. Itaka, Y. Yamasaki, H. Koyama, and K. Kataoka, PEG-detachable polyplex micelles based on disulfide-linked block catiomers as bioresponsive nonviral gene vectors. *J. Am. Chem. Soc.*, **130**, 6001-6009 (2008)
4. T. Noguchi, T. Michihata, W. Nakamura, T. Takumi, R. Shimizu, M. Yamamoto, M. Ikeda, Y. Ohmiya and Y. Nakajima, Dual-color luciferase mouse directly demonstrates coupled expression of two clock genes. *Biochemistry*, **49**, 8053-8061 (2010)
5. Jun Sun, Crystal S. Conn, Yan Han, Vincent Yeung, Shu-Bing Qian, PI3K-MTORC1 attenuates stress response by inhibiting capindependent HSP70 translation. *Journal of Biochemistry*, in press (2011)

Product Name	AB-2550　Kronos Dio
Container	Culture dish, diameter 35 mm
Number of samples	8
Temperature control	Peltier device and forced air circulation
Temperature range in Kronos Dio incubator	From room temperature – 5°C to 45°C in increments of 1°C (Lowest temp.; 20°C)
Temperature control accuracy in Kronos Dio incubator	±0.5°C under room temperature of 25°C
Detector	Photomultiplier tube (PMT)
Measurement method	Photon counting method with a photomultiplier tube
Detectable range of wavelength	350 nm to 670 nm
Measurement time	1 to 60 seconds in increments of 1 second 1 to 60 minutes in increments of 1 minute
Measurement interval	From less than 1 minutes, which depends on time required for eight samples, to 300 minutes in increments of 1 minute
Optical long pass filter	F0: No filter (whole wavelength) F1: O56 filter (cutoff wavelength: less than 560 nm ± 5 nm) F2: R62 filter (cutoff wavelength: less than 620 mm ± 5 nm)
Color separation	Measurement with automatically selected optical filter up to three colors
CO_2 gas precision regulator	Maximum inlet pressure: 1 MPa Pressure range: 0.007 to 0.07 MPa
CO_2 gas controller	Electromagnetic valve Inlet pressure: -27 to 100 kPa Outlet pressure: 0 to 50 kPa
CO_2 gas sensor	Measurement range: 0 to 20% CO_2 (±0.1%)
PC environmental requirements	OS: Windows XP/Vista/7 (32-bit version) Recommended memory: more than 2GB HD: Free space of 1 GB or more
Communication	USB (Version 1.1) interface
Dimension	280(W) × 400(D) × 330(H) mm
Weight	16.0 kg

ATTO Corporation

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

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

e-mail: eig@atto.co.jp

URL: http://www.atto.co.jp/eng/index.html