COSMO BIO CO., LTD.
Inspiration for Life Science

Catalog No. LUC-E02

Luciferase FM Plus
(High sensitivity bioluminescence kit)

Reagent set for the highly sensitive and quantitative bioluminescence detection of ATP.

Introduction

The determination of ATP using bioluminescence is a well-established technique. ATP-depending bioluminescence reaction is used for the measurement of extremely low concentrations of ATP (see Fig.1).
Luciferase FM is a genetically modified firefly luciferase that has the luminous intensity 15 times higher compared to the original North American firefly luciferase. The luciferase FM Plus contains ready-to-use reagent composed of lyophilized luciferase FM, luciferin, and optimized buffer for the highest sensitivity. ATP can be measured with the reagent that is dissolved with the attached ATP-free water by using tube-luminometers as well as microplate readers.

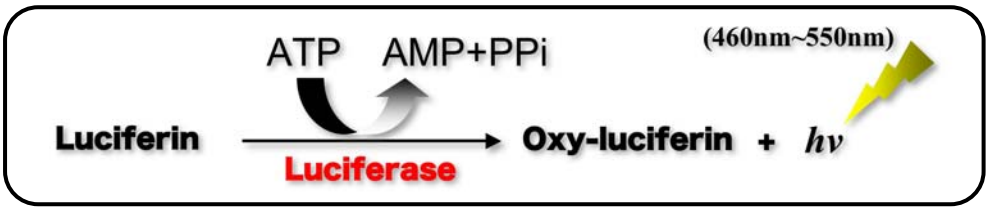

Figure 1 Principal of bioluminescence by firefly luciferse/luciferin

Components:

1. Luciferase reagent, lyophilized (5 mL ×2 bottles, white screw-cap)
2. Sterilized water, ready-to-use (30 mL ×1 bottle, white plastic bottle)

Strage condition

Store 4℃ in the dark (below -18℃ for prolonged storage)
Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

Working range and detection limit

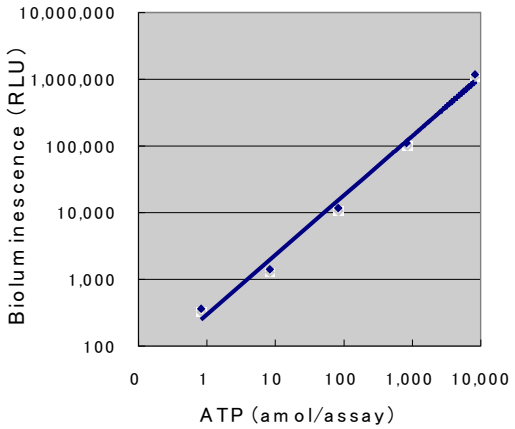

Figure 2 Typical ATP standard curves

The working range of the luciferase FM Plus is between 1 and 10,000 amol ATP.
The detection limit for ATP, using a tube-luminometer, is in the range 1 amol.

Standard protocol

1. Preparation of working solutions and stability

1-1. Luciferase reagent

(1) Dissolve the whole content of one bottle 1 by carefully adding 2 ml of sterilized water.
(2) Incubate for 5 min at room temperature without stirring or shaking. Then mix the solution by pipet up and down carefully.Do not shake!

Note : * Reconstituted luciferase reagent should be divided into aliquots promptly and frozen at -18°C if not used immediately. Each freeze/thaw-cycle reduces the luciferase activity to a certain degree, depending on the freezing conditions (shock freezing is most considerate). Avoid repeated freezing and thawing.

1-2. Sterilized water

The sterilized water supplied with the kit is essentially free of ATP and microbial contaminations. Use this water for reconstitution and dilution of the kit reagents. It can also be used as diluent for samples.

1-3. Working procedure

The standard protocols as described are general guidelines and first choice protocols but are open to variations upon special needs.
Within certain limits the ratios of the assay components can be varied with having little influence on the sensitivity of the assay.

2. Determination of free ATP

2-1. Tube-format protocol]

(1) If necessary, dilute samples with sterilized water to an appropriate ATP concentration. The optimal detection range is between 10^{-12} and 10^{-18} moles ATP (10^{-8} to 10^{-14} M). The pH of the sample has to be in the range of 7.6 to 8.
(2) Dilute ATP standard with sterilized water by serial dilution in the range of 10^{-12} and 10^{-18} moles ATP (10^{-8} to 10^{-14} M).
(3) Add 45 ul luciferase reagent in tube and put on the dark place for 30 seconds over.
(4) Add 5 ul of the samples/standards to luciferase reagent and start measurement for 10 seconds immediately.
(5) Subtract the blank from the raw data and calculate ATP concentrations from a log-log plot of the standard curve data.

2-2. Microplate reader protocol

(1) If necessary, dilute samples with sterilized water to an appropriate ATP concentration. The optimal detection range is between 10^{-10} and 10^{-16} moles ATP (10^{-6} to 10^{-12} M). The pH of the sample has to be in the range of 7.6 to 8.
(2) Dilute ATP standard with sterilized water by serial dilution in the range of 10^{-10} and 10^{-16} moles ATP (10^{-6} to 10^{-12} M).
(3) Put 5 ul of the samples/standards in the microplate reader.
(4) Add 45 ul of luciferase reagent by automated injection and start measurement for 10 seconds immediately.
(5) Subtract the blank from the raw data and calculate ATP concentrations from a log-log plot of the standard curve data.

Experiment example : Measurement of bactirial ATP

1. Preparation of working solutions and stability

See to Standard protocol (1.).

2. Preparation of bacterial sample

(1) Collect bacterium suspension to 1.5mL tube, centrifugation (15,000 rpm, 3 min, RT) and discard the supernatant.
(2) Add 0.5 ml of sterilized water to pellet, tap the tube (suspend pellet), centrifugation (15,000 rpm, 3 min, RT) and discard the supernatant (Washing of bacterium).
(3) Add 450 ul of sterilized water to pellet, tap the tube (suspend pellet), add 50 uL of ATP extract (0.5 % benzalkonium chloride, sterilized water) and put for 5 min at RT. This suspension is a measurement solution (Test sample).

3. Determination of free ATP

See to Standard protocol (2.).

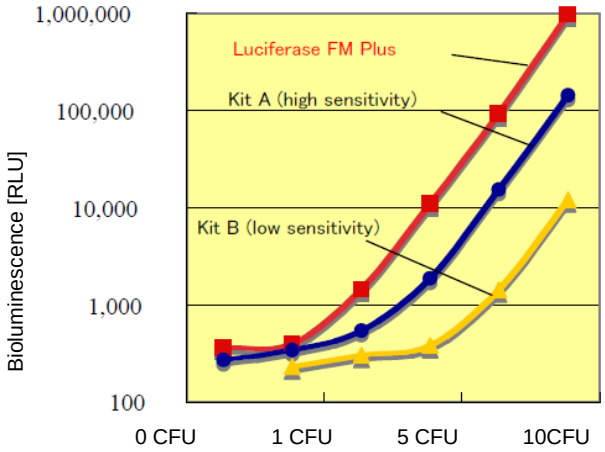

Figure 3 Bacterial cells number (CFU/assay)
Measurement of diluted *E. coli* culutre.
Luciferase FM Plus can be used for the detection of intracellular ATP extracted from a single bacterial cell.

References

1. Fujii, H , et al., *Anal Biochem.* 2007 Jul 15;366(2):131-6. PMID:17540326
2. Noda, K., et al., *Biotechnol Lett.* 2008 Jun;30(6):1051-4. PMID:18224280
3. Noda, K., et al., *Anal Biochem*. 2010 Feb 15;397(2):152-5. PMID:19850001

RELATED PRODUCTS:

Product Name	Quantity	Maker	Cat#
LUCIFERASE FM Highly Sensitive Firefly Luciferase. Luciferase FM has the luminous intensity 15 times or more general luciferase.	10*1 mg	CSR	LUC-E01
LUCIFERASE FM PLUS Reagent set for the highly sensitive and quantitative detection of ATP by luciferase driven bioluminescence.	10*80 Test	CSR	LUC-E02

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COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
URL: http://www.cosmobio.co.jp e-mail: export@cosmobio.co.jp
[Outside Japan] Phone : +81-3-5632-9617 [国内連絡先] Phone : +81-3-5632-9610
FAX : +81-3-5632-9618 FAX : +81-3-5632-9619