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Revised: February 27, 2013

Product Information

ATP-Glo™ Bioluminometric Cell Viability Assay Kit

Catalog Number: 30020-T, 30020-1, 30020-2

Unit Size:
30020-T: 50 assays
30020-1: 200 assays
30020-2: 1000 assays

Kit Contents

Component	30020-T 50 assays	30020-1 200 assays	30020-2 1000 assays
D-Luciferin	2 x 1 mg (99907)	8 x 1 mg (99907)	4 x 10 mg (99908)
Firefly Luciferase	1 x 50 uL (99984)	1 x 200 uL (99930)	5 x 200 uL (99930)
Assay Buffer	5 mL (30020CT)	2 x 10 mL (30020C1)	1 x 100 mL (30020C2)
ATP (2 mM)	1 x 100 uL (99931)	1 x 100 uL (99931)	1 x 100 uL (99931)

Storage and Handling

Store the ATP-Glo Cell Viability Assay Kit at –70°C. Avoid repeated freeze-thaw cycles. Product is stable for at least 6 months from date of receipt when stored as recommended.

Product Description

ATP-Glo™ Cell Viability Assay offers a highly sensitive homogenous assay for quantifying ATP. The homogeneous assay procedure involves a single addition of ATP-Glo detection cocktail directly to cells cultured in a serum-supplemented medium. No cell washing, medium removal and multiple pipetting are required.

Because ATP is an indicator of metabolically active cells, the number of viable cells can be assessed based on the amount of ATP available. This ATP detection kit takes advantage of Firefly luciferase's use of ATP to oxidize D-Luciferin and the resulting production of light in order to assess the amount of ATP available (Fig. 1).

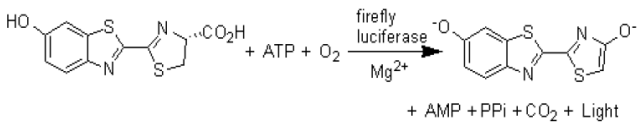

Figure 1. Bioluminescent reaction catalyzed by Firefly luciferase.

The ATP-Glo kit can be used to detect as little as a single cell or 0.01picomoles of ATP. The signal produced is linear within 6 orders of magnitude (Fig. 2A). By relating the amount of ATP to the number of viable cells, the assay has wide applications, ranging from the determination of viable cell numbers to cell proliferation to cell cytotoxicity (Fig. 2B).

ATP-Glo is a flash-type luminescence assay designed for individual sample detection by using a luminometer in a single sample format or a luminometer with an injector in 96-well plate format. The luminescence signal generated is stable for about 1 minute.

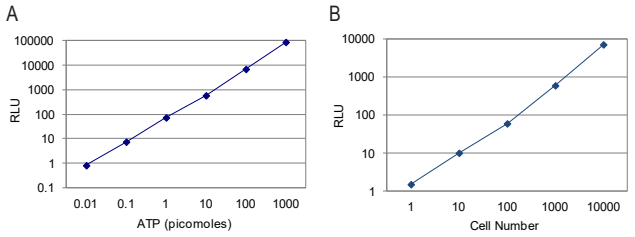

Figure 2. Luminescence dose response curves. A. Ten-fold dilutions of ATP were prepared in 100 uL PBS per sample. B.Ten-fold dilutions of Jurkat cells were prepared in 100 uL RPMI medium per sample. Immediately before luminescence measurement, 100 uL of ATP-Glo detection cocktail was added to each sample. Luminescence was measured for 10 seconds using a Turner Designs single tube luminometer.

Assay Procedure

A. Sample Preparation

1. Prepare each sample in 100 uL dH_2O, PBS, or cell culture medium for the assay.

2. For quantifying absolute ATP amount, a series of ATP standards can be prepared in 100 uL of the same diluent as the samples (Table 1).

   Note: Higher luminescence signal is obtained for samples prepared in dH_2O than in medium or PBS. Maximum signal is obtained when sample is prepared in 100 uL dH_2O. For samples in PBS or cell culture medium, signal can be increased by preparing the sample in 10 uL or lower volume and subsequently diluting to 100 uL with dH_2O. For quantifying absolute ATP amount, a series of ATP standards can be made in 10 uL of PBS or medium and diluted to 100 uL with dH_2O (Table 2).

Table 1. Preparation of ATP standards by serial dilution in dH_2O, PBS, or cell culture medium.

	Volume of ATP solution	Volume of diluent	Final ATP concentration	ATP per 100 uL*
A	2.5 uL 2 mM ATP standard (99931)	500 uL	10 uM	1000 pmol
B	50 uL solution A	450 uL	1 uM	100 pmol
C	50 uL solution B	450 uL	100 nM	10 pmol
D	50 uL solution C	450 uL	10 nM	1 pmol
E	50 uL solution D	450 uL	1 nM	0.1 pmol
F	--	500 uL	0	0 pmol

* Transfer 100 uL of each ATP standard to a fresh tube for assay.

Table 2. Preparation of ATP standards in 10 uL PBS or cell culture medium for dilution to 100 uL with dH_2O.**

	Volume of ATP solution	Volume of diluent	Final ATP concentration	ATP per 10 uL**
A	2.5 uL 2 mM ATP standard (99931)	50 uL	100 uM	1000 pmol
B	5 uL solution A	45 uL	10 uM	1000 pmol
C	5 uL solution B	45 uL	1 uM	100 pmol
D	5 uL solution C	45 uL	100 nM	10 pmol
E	5 uL solution D	45 uL	10 nM	1 pmol
F	--	50 uL	0	0 pmol

** Before assay, transfer 10 uL of each ATP standard to a fresh tube. Add 90 uL dH_2O to each tube and mix well.

B. Preparation of ATP-Glo Detection Cocktail

1. Thaw a bottle of ATP-Glo Assay Buffer and pipette a desired volume (2.5 mL or 25 mL) from the bottle into a new container.

2. In a clean container, dissolve the supplied D-luciferin with the above Assay Buffer to prepare a final concentration of 0.4 mg/mL. Each 1 mg D-luciferin vial can make 2.5 mL of assay solution, and each 10 mg vial of D-luciferin can make 25 mL of assay solution.

   Note: If you need less than 2.5 mL or 25 mL ATP-Glo assay solution as described in step 2, you may prepare a 25X (10 mg/mL) D-Luciferin stock solution in dH_2O and store it at -20°C or below for repeated use. The D-luciferin stock solution should be stable for at least one month, depending on the frequency of freeze-thaw cycles. A desired volume of the ATP-Glo assay solution can be prepared by diluting the D-Luciferin stock solution 1:25 in ATP-Glo Assay Buffer for a final concentration of 0.4 mg/mL D-luciferin.

3. Add Firefly Luciferase to the ATP-Glo assay solution in a ratio of 1 uL to 100 uL (25 uL Luciferase for 2.5 mL or 250 uL Luciferase for 25 mL of the ATP-Glo assay solution). ATP-Glo Detection Cocktail should be prepared fresh before each use for maximum activity.

C. Luminescence measurement

   Note: Luminescence can be measured using a single sample luminometer with or without an injector or a 96-well plate luminometer with an injector.

1. Set up your luminometer with a delay time of 0-10 seconds, an integration time of 10 seconds, and the appropriate sensitivity. For manual addition, we recommend setting the delay time to 0. For automated injection, we recommend setting a delay time of 5-10 seconds to allow sample to reach equilibrium.

2. Add or inject 100 uL of ATP-Glo Detection Cocktail into a sample.

3. Mix quickly by flicking the tube with a finger for thorough mixing (manual addition).

4. Place tube in luminometer and initiate measurement (manual addition).

5. Measure the luciferase activity for 10 seconds.

6. Discard the used reaction tube or skip the used well and proceed to the next sample.

7. Repeat steps 2-5 for each additional sample.

References

1. Kangas L. et al. 1984. Bioluminescence of cellular ATP: a new method for evaluating agents in vitro. Medical Biology, 62, 338-343.
2. Lundin A. et al. 1986. Estimation of biomass in growing cell lines by ATP assay. Methods Enzymol. 133, 27-42
3. Crouch S.P.M., et al. 1993. The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. J. Immunol Methods, 160, 81-88.
4. Petty R.D., et al. 1995. Comparison of MTT and ATP-based assays for the measurement of viable cell number. J. Biolumin. Chemilumin. 10, 29-34
5. Cree I.A. and Andreotti P.E. 1997. Measurement of cytotoxicity by ATP-based luminescence assay in primary cell cultures and cell lines. Toxicology in Vitro, 11, 553-556.

Related Products

Catalog number	Product
30026	Calcein AM Cell Viability Assay Kit
30002	Viability/Cytotoxicity Assay for Animal Live & Dead Cells
30025	Resazurin Cell Viability Assay Kit
30006	MTT Cell Viability Assay Kit
30007	XTT Cell Viability Assay Kit
30050	CFDA-SE Cell Viability Assay Kit
30027	Viability/Cytotoxicity Assay for Bacteria Live & Dead Cells
32001	Bacterial Viability and Gram Stain Kit
30003	Firefly Luciferase Assay Kit
30004	*Renilla* Luciferase Assay Kit
30005	Firefly & *Renilla* Luciferase Assay Kit
30028	Steady-Luc™ Firefly HTS Assay Kit

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