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Biotium, Inc.
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ATP-Glo™ Bioluminometric Cell Viability Assay Kit

Catalog Number: **30020-1** (200 assays)
30020-2 (1000 assays)

Contact Information

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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.

Since 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.

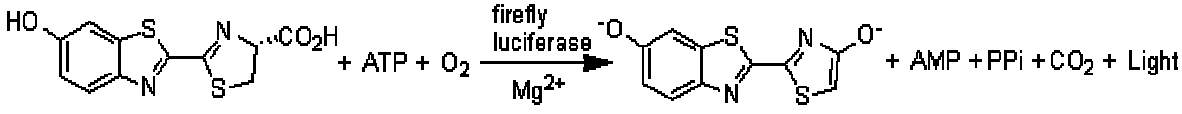

Figure 1. Bioluminescent reaction catalyzed by *Firefly* luciferase.

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 (Figure 2). 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 (Figure 3).

This assay is 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 within 1 minute.

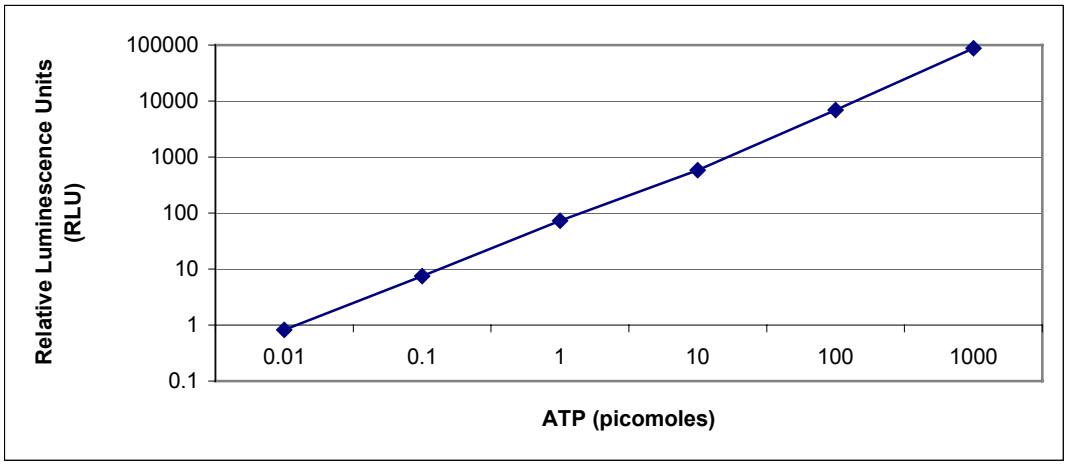

Figure 2. ATP Dose Response Curve. A series of ten-fold titrations from 1000 picomoles to 0.01 picomoles of ATP were prepared in 100 uL PBS for each sample in a 1.5 mL microfuge tube. 100 uL of ATP-Glo™ detection cocktail (see section B of assay procedure) was then added into each microfuge tube containing indicated amount of ATP. The microfuge tube was flicked for a few times for thorough mixing and placed into a luminometer(Turner Designs) immediately for luminescence signal detection. Light emission was integrated over 10 seconds with 5 second pre-read delay. Both RLU and ATP amount are in log scales.

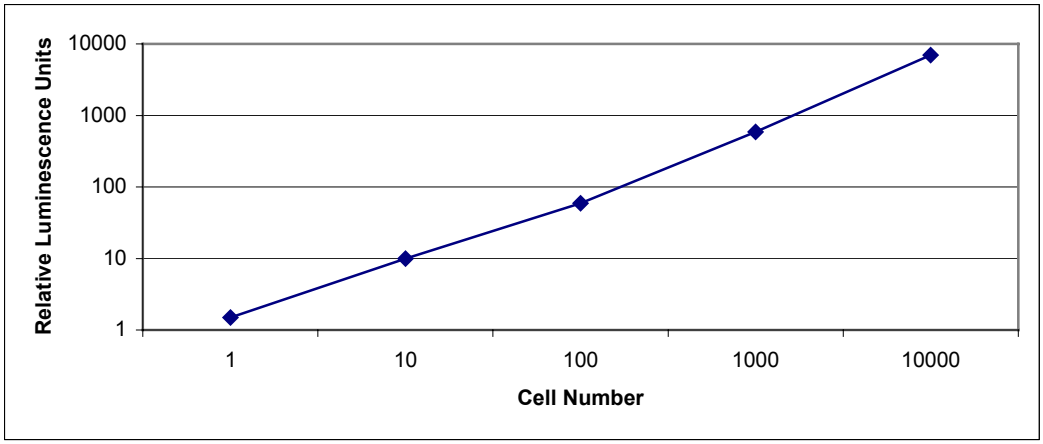

Figure 3. Cell Number Titration Curve. A series of ten-fold titrations of Jurkat cells (1-10,000) were prepared in 100 uL RPMI medium in 1.5 mL microfuge tubes. 100 uL of ATP-Glo™ detection cocktail (see section B of assay procedure) was then added into each microfuge tube containing various number of Jurkat cells. The microfuge tube was flicked for a few times for thorough mixing and placed into a luminometer(Turner Designs) immediately for luminescence signal detection. Light emission was integrated over 10 seconds with 5 second pre-read delay. Both RLU and cell number are in log scales.

Product Components

ATP-Glo™ Bioluminometric Cell Viability Assay Kit, 30020-1 (200 assays)

8 x 1mg D-Luciferin
1 x 200uL *Firefly* Luciferase
2 x 10mL ATP-Glo™ Assay Buffer
1 x 100uL ATP (2mM)
1 Protocol

ATP-Glo™ Bioluminometric Cell Viability Assay Kit, 30020-2 (1000 assays)

4 x 10mg of D-Luciferin
5 x 200uL *Firefly* Luciferase
1 x 100mL ATP-Glo™ Assay Buffer
1 x 100uL ATP (2mM)
1 Protocol

Storage Conditions

Store ATP-Glo™ Cell Viability Assay Kit at –70°C. Avoid repeated freeze-thaw cycles.

Assay Procedure

A. Sample Preparation

Prepare each sample in 100uL medium or 100uL PBS for the assay. For quantifying ATP amount instead of cell number, sample can be prepared in 100 uL DI H_2O.

Note: Higher luminescence signal is obtained for samples prepared in DI H_2O than in medium or PBS. For quantifying absolute ATP amount, a series of ATP dilutions serving as ATP standards should also be prepared in the same solution and in the same volume as samples. Sample may also be prepared in 10 uL or less volume. Maximum signal is obtained when sample is prepared in 100 uL DI H2O or 10 uL or less buffered solution such as PBS and medium.

B. Preparation of ATP-Glo™ Detection Cocktail

1. Thaw a bottle of ATP-Glo™ Assay Buffer and pipette a desired volume (2.5mL or 25mL) 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 0.4mg/mL. Each 1mg D-luciferin vial can make 2.5mL of assay solution, and each 10mg vial of D-luciferin can make 25mL of assay solution.

Note: If you need less than 2.5mL or 25mL ATP-Glo™ assay solution as described in step 2, you may dissolve D-Luciferin in 100 uL DI water as 10 mg/mL stock solution 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 cycle. A desired volume of the ATP-Glo™ assay solution can be prepared by diluting the 25X D-Luciferin stock solution with the ATP-Glo™ Assay Buffer to 0.4mg/mL D-luciferin.

3. Add *Firefly* Luciferase to the ATP-Glo™ assay solution in a ratio of 1uL to 100 uL (25uL 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. Assay Protocol

The assay can be done by 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 mode, we recommend setting the delay time to 0. For automotive injection, we recommend setting a delay time of 5-10 seconds to allow sample to reach equilibrium.

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

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

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

5. Measure the luciferase activity.

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

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2.Lundin A. et al. 1986. Estimation of biomass in growing cell lines by ATP assay. *Methods Enzymol.* 133, 27-42
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