ATP Cell Viability Luciferase Assay

Cell Based Assay

Cat. # SCT149

pack size: 1 Kit

Store at -80ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

Because ATP is an indicator of metabolically active cells, the number of viable cells can be assessed based on the amount of ATP available. The ATP Cell Viability Luciferase Assay offers a highly sensitive homogenous assay for quantifying ATP in cell cultures. This 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 in cell cultures. The sensitive assay procedure involves a single addition of ATP detection cocktail directly to cells cultured in a serum-supplemented medium. No cell washing, medium removal and multiple pipetting are required. The kit can be used to detect as little as a single cell or 0.01 picomoles of ATP. The signal produced is linear within 6 orders of magnitude. 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.

The ATP Cell Viability Luciferase Assay 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.

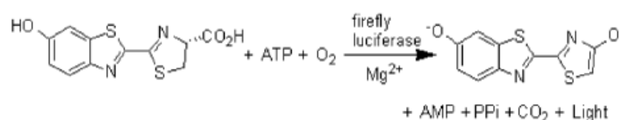

Figure 1. Assay principle. Firefly luciferase's use of ATP to oxidize D-Luciferin and the resulting production of light in order to assess the amount of ATP available

Storage

Store ATP Cell Viability Luciferase Assay at -80°C. Avoid repeated freeze-thaw cycles. Product is stable for at least 6 months from date of receipt when stored as recommended.

Measurement of Cell Viability using the ATP Cell Viability Luciferase Assay

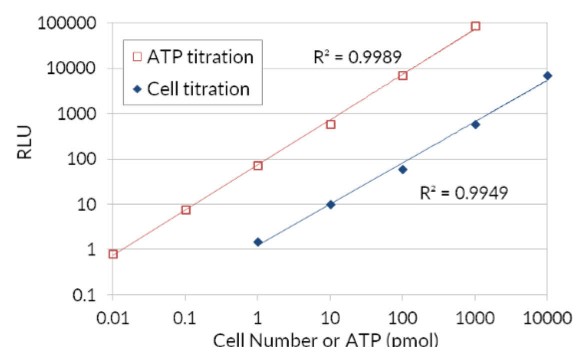

Figure 2. **Luminescence dose response curves**. A) Ten-fold dilutions of ATP were prepared in 100 uL PBS per sample. B) Ten-fold dilutions of Jurkat cells were prepared in 100 uL RPMI medium per sample. Immediately before luminescence measurement, 100 uL of ATP detection cocktail was added to each sample.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

Kit Components

1) D-Luciferin (CS224519): 4 X 10 mg
2) Firefly Luciferase (CS224520): 5 X 200 µL
3) ATP Assay Buffer (CS224521): 1 X 10 mL
4) ATP, 2µM (CS224522): 1 X 100 µL

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Assay Protocol

Sample Preparation

1. Prepare each sample in 100 uL dH2O, 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 dH2O than in medium or PBS. Maximum signal is obtained when sample is prepared in 100 uL dH2O. 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 dH2O. 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 dH2O (Table 2).

	Volume of ATP Solution	Volume of Diluent	Final ATP Concentration	ATP per 100 uL*
A	2.5 uL, 2 mM ATP Standard	500 µL	10 µM	1000 pmol
B	50 uL solution A	450 µL	1 µM	100 pmol
C	50 uL solution B	450 µL	100 nM	10 pmol
D	50 uL solution C	450 µL	10 nM	1 pmol
E	50 uL solution D	450 µL	1 nM	0.1 pmol
F	N/A	500 µL	0	0

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

Table 1. Preparation of ATP standards by serial dilution

	Volume of ATP Solution	Volume of Diluent	Final ATP Concentration	ATP per 100 uL**
A	2.5 uL, 2 mM ATP standard	50 µL	100 µM	1000 pmol
B	5 uL solution A	45 µL	10 µM	1000 pmol
C	5 uL solution B	45 µL	1 µM	100 pmol
D	5 uL solution C	45 µL	100 nM	10 pmol
E	5 uL solution D	45 µL	10 nM	1 pmol
F	N/A	50 µL	0	0

** Before assay, transfer 10 uL of each ATP standard to a fresh tube. Add 90 uL dH2O to each tube and mix well.

Table 2. Preparation of ATP standards in 10 uL PBS or cell culture medium for dilution to 100 uL with dH2O.

Preparation of ATP Detection Cocktail

1. Thaw a bottle of ATP 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 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 assay solution can be prepared by diluting the D-Luciferin stock solution 1:25 in ATP Assay Buffer for a final concentration of 0.4 mg/mL D-luciferin.

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

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Luminescence Assay

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 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.

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