Bioluminescent Cell Viability Kit I (ATP)

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K254-200
Description	Cell death (especially apoptosis) is an energy-dependent process that requires ATP. As ATP levels fall to a point where the cell can no longer perform basic metabolic functions, the cell will die. A typical apoptotic cell exhibits a significant decrease in ATP level. Therefore, loss of ATP level in cell has been used as an indicator of cell death. In contrast, cell proliferation has been recognized by increased levels of ATP. The PromoKine Bioluminescent Cell Viability Kit I utilizes bioluminescent detection of the ATP levels for a rapid screening of apoptosis and cell viability/proliferation simultaneously in mammalian cells. 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 assay utilizes Firefly luciferase to catalyze the formation of light from ATP and D-Luciferin, and the produced light can be measured using a luminometer or Beta Counter in order to assess the amount of ATP available.

$$\text{Luciferin} + \text{ATP} + O_2 \xrightarrow[\text{Luciferase}]{Mg^{2+}} \text{Oxyluciferin} + \text{AMP} + \text{Pyrophosphate} + CO_2 + \text{Light}$$

This is 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. The assay can be fully automatic for high throughput (10 seconds/sample) and is extremely sensitive (detects 10-100 mammalian cells/well). It 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 high sensitivity of this assay has led to many other applications for detecting ATP production in various enzymatic reactions, as well as for detecting low level bacterial contamination in samples such as blood, milk, urine, soil, and sludge.

The Bioluminescent Cell Viability Kit I (ATP) 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.

Quantity: 200 assays

Kit Components

Components	**Quantity** *PK-CA577-K254-200*	*PK-CA577-K254-1000*
Firefly Luciferase	1 x 200 µl	5 x 200 µl
Assay Buffer	2 x 10 ml	1 x 100 ml
D-Luciferin	8 x 1 mg	4 x 10 mg
ATP (2 mM)	1 x 100 µl	1 x 100 µl

Applications / Assay Protocol

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 µl of the same diluent as the samples (Table 1).

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 µl*
A	2.5 µl 2 mM ATP standard	500 µl	10 µM	1000 pmol
B	50 µl solution A	450 µl	1 µM	100 pmol
C	50 µl solution B	450 µl	100 nM	10 pmol
D	50 µl solution C	450 µl	10 nM	1 pmol
E	50 µl solution D	450 µl	1 nM	0.1 pmol
F	--	500 µl	0	0 pmol

* Transfer 100 µl 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 µl with dH_2O**

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

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

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 µl dH_2O. For samples in PBS or cell culture medium, signal can be increased by preparing the sample in 10 µl or lower volume and subsequently diluting to 100 µl with dH_2O. For quantifying absolute ATP amount, a series of ATP standards can be made in 10 µl of PBS or medium and diluted to 100 µl with dH_2O (Table 2).

B. Preparation of *ATP Detection Cocktail*

1. Thaw a bottle of 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 *ATP 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 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 µl to 100 µl (25 µl Luciferase for 2.5 ml or 250 µl Luciferase for 25 ml of the *ATP assay solution*). *ATP 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 µl 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.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

Storage & Stability

Store kit at –70°C upon arrival. Store individual reagents as indicated on the respective labels. Avoid repeated freeze-thaw cycles. Product is stable for at least 6 months from date of receipt when stored as recommended.