Bioluminescent Cell Viability Kit II (ADP/ATP)

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K255
Description	The changes in ADP/ATP ratio have been used to differentiate the different modes of cell death and viability. Increased levels of ATP and decreased levels of ADP have been recognized in proliferating cells. In contrast, decreased levels of ATP and increased levels of ADP are recognized in apoptotic cells. The decrease in ATP and increase in ADP are much more pronounced in necrosis than apoptosis. The PromoKine Bioluminescent Cell Viability Kit II (ADP/ATP) utilizes bioluminescent detection of the ADP and ATP levels for a rapid screening of apoptosis, necrosis, growth arrest, and cell proliferation simultaneously in mammalian cells. The assay utilizes the enzyme luciferase to catalyze the formation of light from ATP and luciferin, and the light can be measured using a luminometer or Beta Counter. ADP level is measured by its conversion to ATP that is subsequently detected using the same reaction. The assay can be fully automatic for high throughput and is highly sensitive (detects 100 mammalian cells/well).
Quantity	200 assays

Kit Components

Components	Quantity	Cap Code
Nucleotide Releasing Buffer	50 ml	NM
ATP Monitoring Enzyme (green cap)	1 vial	Green
ADP Converting Enzyme (blue cap)	1 vial	Blue
Enzyme Reconstitution Buffer (clear cap)	2 ml	Red

Applications / Assay Protocol

A. Reagent Reconstitution and General Consideration:

Reconstitute ATP Monitoring Enzyme with 2 ml of the Enzyme Reconstitution Buffer and mix gently by inversion.

Reconstitute ADP Converting Enzyme with 220 µl of the Nucleotide Releasing Buffer and mix gently by inversion.

The reconstituted enzymes are stable for up to 2 months at 4°C after reconstitution. For more accurate handling, the reconstituted ADP-Converting Enzyme can be diluted 10-fold with Nucleotide Releasing Buffer just before use (Section B, Step 7), then use 10 µl of the enzyme for each assay.

The Bioluminescent Cell Viability Kit II kit is significantly more sensitive than other methods used for cell viability assays. The method can detect as few as 10 cells, but as a general guide, we recommend using 1 x 10^4 cells per assay. Avoid contamination with ATP from exogeneous biological sources, such as bacteria or fingerprints.

Ensure that the Nucleotide Releasing Buffer is at room temperature before use. The optimal temperature is 22°C. Keep other enzymes on ice during the assay and protect from light as much as possible.

The assay can be performed using either a single tube or a white walled 96-well luminometer plate (100 µl/well culture volume is recommended).

B. Assay Protocol:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.

2. For each sample well to be measured, mix 100 µl of *Reaction Mix* consisting of:
   - ATP monitoring enzyme: 10 µl
   - Nucleotide Releasing Buffer: 90 µl

3. Add 100 µl of the *Reaction Mix* to the appropriate wells of a 96-well plate and read the background luminescence (Data A). For higher accuracy let the reaction mix sit at room temperature to burn off low level ATP contamination for a few hours.

4. For suspension cells, transfer 10 µl of the cultured cells (10^3 – 10^4) to the appropriate wells of the luminometer plate containing the *Reaction Mix* (from Step 3).

5. For adherent cells, remove culture medium and treat cells (10^3 – 10^4) with 50 µl of Nucleotide Releasing Buffer for 5 minutes at room temperature with gentle shaking. Then, transfer it to the appropriate wells of the luminometer plate containing the *Reaction Mix* (from Step 3).

6. After ~2 minutes, read the sample in a luminometer or luminescence-capable plate reader (Data B).

7. To measure ADP levels in the cells, read the samples (from step 6) again (Data C), then add 1 µl of ADP Converting Enzyme. Read the samples after ~2 minutes (Data D).

Note: The results can be analyzed using cuvette-based luminometers or Beta Counters. When Beta Counter is used it should be programmed in the "out of coincidence" (or Luminescence mode) for measurement. The entire assay can directly be done in a 96-well plate*. It can also be programmed automatically using instrumentation with injectors (When using injector the ATP Monitoring Enzyme and the ADP Converting Enzyme can be diluted with the Nuclear Releasing Buffer at 1:50 for injector).
*The assay utilizes a "glow-type" luciferase which has replaced the original "flash-type" luciferase. While still sensitive to sub-picomole amounts of ATP, the glow-type reactions can still be read an hour later. This means that ATP & ADP levels are now determined by the steady-state light output levels. This makes the reading of an entire 96-well (384-well) plate much more feasible.

C. Calculation of ADP/ATP Ratio:

ADP/ATP Ratio is calculated as: (Data D – Data C)/(Data B – Data A)

Interpretation of Results:

Cell Fate	ADP Level	ATP Level	ADP/ATP
Proliferation	Very Low	High	Very Low
Growth Arrest	Low	Slightly Increased	Low
Apoptosis	High	Low	High
Necrosis	Much Higher	Very Low	Much Higher

The interpretation of different ratios obtained may vary significantly according to the cell types and conditions used. However, the following criteria may be used as guidelines:

a. Test gives markedly elevated ATP values with no significant increase in ADP levels in comparison to control cells = proliferation.

b. Test gives similar or slightly higher levels of ATP and with little or no change in ADP compared to control = growth arrest.

c. Test gives lower levels of ATP to control but shows an increase in ADP = apoptosis.

d. Test gives considerable lower ATP levels than control but greatly increased ADP = necrosis.

Intended Use

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

Storage & Stability

Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promokine.info
www.promokine.info

North America
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40