Technical Bulletin

Cytotoxicity Assay Kit

Catalogue number MAK536

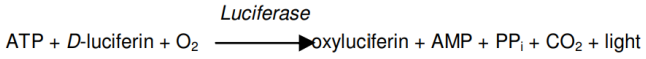
Product Description

Adenosine 5'-triphosphate (ATP) is the chemical energy for cellular metabolism and is often referred to as "energy currency" of the cell. ATP is produced only in living cells during photosynthesis and cellular respiration and consumed in cellular processes including biosynthetic reactions, motility and cell division. It is a key indicator of cellular activity and has been utilized as a measure of cell viability and cytotoxicity in research and drug discovery.

The Cytotoxicity Assay Kit provides a rapid method to measure intracellular ATP, cell viability and cytotoxicity.

The single working reagent lyses cells to release ATP, which, in the presence of luciferase, immediately reacts with the Substrate D-luciferin to produce light. The light intensity is a direct measure of intracellular ATP concentration and hence number of living cells.

This non-radioactive, homogeneous cell-based assay can be conveniently performed in microplates. The reagent is compatible with all culture media and liquid handling systems for high-throughput screening applications in 96-well and 384-well plates. This kit can detect as low as 50 cells in a given sample. The kit is used to study effects of cytokines, growth factor, nutrients on cells.



Components

The kit is sufficient for 100 bioluminescent assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK536A
- Substrate 120 µL
  Catalogue Number MAK536B
- ATP Enzyme 120 µL
  Catalogue Number MAK536C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example., multichannel pipettor)
- Luminometer.
- White opaque tissue culture plates.
- 1.5 mL microcentrifuge tubes
- PBS (Catalogue number P4474 or equivalent)
- Cell Culture Medium.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Keep thawed ATP Enzyme on ice or 4 °C.

Note: After adding the Reconstituted Reagent, the luminescence signal is stable for about 15 min and decreases slow thereafter. Reading is best performed within 30 min.

Procedure

All Samples and Standards should be run in duplicate.

Assay Procedure In 96-Well Plates

Cell Culture

1. Plate cells at 100 µL/well in white opaque tissue culture plates.
2. If desired, add 5 µL test compounds and controls dissolved in PBS or culture medium per well.
3. Rock plate lightly to mix and incubate for desired period of time (example, overnight).

Assay Reaction

Prepare Working Reagent as per Table 1. Each well requires 90 µL of Working Reagent. Prepare enough for the assay.

Table 1.
96-well Working Reagent Preparation

Reagent	Volume (µL)
Assay Buffer	95
Substrate	1
ATP Enzyme	1

1. Add 90 µL Reconstituted Reagent to each test well.
2. Tap the plate to mix well.
3. Incubate for 2 minutes at room temperature.
4. Read luminescence on a luminometer.

Note: For most luminometers (Berthold Luminometer, LJL Analyst, Top Count, MicroBeta Counters, CLIPR and LeadSeeker), integration time of 0.1 to 5 sec is appropriate.

Assay Procedure In 384-Well Plates

Cell Culture

1. Plate cells at 25 µL/well in white opaque tissue culture plates.
2. If desired, add 5 µL test compounds and controls dissolved in PBS or culture medium per well.
3. Rock plate lightly to mix and incubate for desired period of time (example, overnight).

Assay Reaction

Prepare Working Reagent as per Table 2. Each well requires 25 µL of Working Reagent. Prepare enough for the assay.

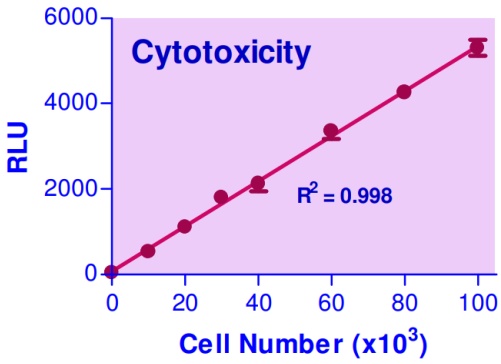
Table 2.
384-well Working Reagent Preparation

Reagent	Volume (µL)
Assay Buffer	30
Substrate	0.3
ATP Enzyme	0.3

1. Add 25 µL Reconstituted Reagent to each test well.
2. Tap the plate to mix well.
3. Incubate for 2 minutes at room temperature.
4. Read luminescence on a luminometer.

Note: For most luminometers (Berthold Luminometer, LJL Analyst, Top Count, MicroBeta Counters, CLIPR and LeadSeeker), integration time of 0.1 to 5 sec is appropriate.

Figure 1.
Typical Linearity of Luminescence in 96 well plate



References

1. Li W., *et al.,* Human primary renal cells as a model for toxicity assessment of chemo-therapeutic drugs. *Toxicol In Vitro*., **20(5)**, 669-76 (2006).
2. Zhelev Z., *et al.,* Phenothiazines suppress proliferation and induce apoptosis in cultured leukemic cells without any influence on the viability of normal lymphocytes. Phenothiazines and leukemia. *Cancer Chemother Pharmacol*., **53(3)**, 267-75 (2004).
3. Ingram PR., *et al.,* A comparison of the effects of ocular preservatives on mammalian and microbial ATP and glutathione levels. *Free Radic Res*., **38(7)**, 739-50 (2004).

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