ApoSENSOR™ Cell Viability Assay Kit

Manufactured by BioVision.

ALX-850-247-KI01: ~200 tests
ALX-850-247-KI02: ~1000 tests

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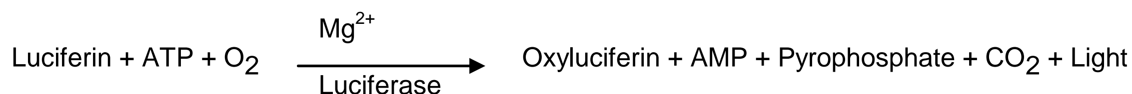
For laboratory use only. Not for human or diagnostic use.

I. Introduction:

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 **ApoSENSOR™ Cell Viablity Assay Kit** utilizes bioluminescent detection of the ATP levels for a rapid screening of apoptosis and cell proliferation simultaneously in mammalian cells. The assay utilizes luciferase to catalyze the formation of light from ATP and luciferin, and the light can be measured using a luminometer or Beta Counter.



The assay can be fully automatic for high throughput (10 sec./sample) and is extremely sensitive (detects 10-100 mammalian cells/well). 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.

II. Kit Contents:

Component	850-247-KI01	850-247-KI02
	200 tests	1000 tests
Nucleotide Releasing Buffer	20 ml	100 ml
ATP Monitoring Enzyme	1 vial	5 vials
Enzyme Reconstitution Buffer	240 µl	1.2 ml
ATP (MW 551)	1 mg	1 mg

III. ApoSENSOR™ Cell Vialbility Assay Protocol:

A. Reagent Reconstitution and General Considerations:

- Reconstitute ATP Monitoring Enzyme with 220 µl/vial of the Enzyme Reconstitution Buffer. Mix well by gentle pipeting. This results in a yellow-green milky-like solution (not clear solution). Aliquot enough enzyme (1 µl per assay) for the number of assays to be performed in each experiment and freeze at -80°C for future use. The reconstituted enzyme is stable for up to 6 months at -80°C.
- Protect the ATP Monitoring Enzyme from light as much as possible.
- Prepare an ATP standard solution by dissolving the 1 mg ATP into 1 ml of H_2O. The solution is stable for several weeks at -20°C.
- The ApoSENSOR™ 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 10^3 –10^4 cells per assay.

- Because of the high sensitivity of the ATP assay, avoid contamination with ATP from exogenous 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 ATP Monitoring Enzyme on ice during the assay.
- 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. Sample Assay Protocol:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
2. For suspension cells, transfer 10 µl of the cultured cells (containing 10^3 –10^4 cells) into luminometer plate. Add 100 µl of the Nuclear Releasing Reagent.
   For adherent cells, remove culture medium and treat cells (10^3 –10^4) with 100 µl of Nuclear Releasing Reagent for 5 minutes at room temperature with gentle shaking.
3. Add 1 µl ATP Monitoring Enzyme into the cell lysate. Read the sample in 1 minute in a luminometer.
4. Fold-decrease (or increase in the case of cell proliferation) in ATP levels can be determined by comparing these results with the levels of uninduced control.

   Note: The assay can be analyzed using cuvet-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 also be done directly in a 96-well plate. It can also be programmed automatically using instrumentation with injectors (When using injector the ATP Monitoring Enzyme can be diluted with the Nuclear Releasing Buffer at 1:50 for injector).

C. Standard Curve:

If the absolute ATP amount in samples needs to be calculated, an ATP standard curve should be generated (using the ATP standard provided in the kit) together with the above assays. Add 10 µl of a series of dilutions of ATP (e.g., 1, 0.1, 0.001, 0.0001, 0.00001, 0.000001 mg/ml, etc. Also includes a 0 mg/ml sample to measure background luminescence) to luminometer plates, then add 100 µl of Nuclear Releasing Reagent and 1 µl of ATP Monitoring Enzyme. Read the samples in 1 minutes in a luminometer (as described above). The background luminescence should be subtracted from all readings. The amount of ATP in uninduced and induced experimental samples can then be calculated from the standard curve.