ApoSENSOR™ Cell Viability Assay Kit

(Catalog #ALX-850-247; Store kit at –20˚C)

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 Viability Vssay 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.

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

The assay can be fully automatic for high throughput (10 seconds/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	200 assays	Part Number
Nucleotide Releasing Buffer	20 ml	ALX-K254-200-1
ATP Monitoring Enzyme	1 vial	ALX-K254-200-2
Enzyme Reconstitution Buffer	2.15 ml	ALX-K254-200-3
ATP (MW 551)	1 mg	ALX-K254-200-4

III. ApoSENSOR™ Cell Viability Assay Protocol:

Á. Reagent Reconstitution and General Consideration:

- Reconstitute ATP Monitoring Enzyme with 2.1 ml/vial of the Enzyme Reconstitution Buffer. Mix well by gentle pipeting. The reconstituted enzyme is stable for up to 2 months at 4˚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 1 x 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 10 µl ATP Monitoring Enzyme into the cell lysate. Read the sample in ~1-2 minutes 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 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 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 1:4 for injector. Mix a solution to the ratio of 10 µl ATP monitoring enzyme: 40 µl of Nucleotide Releasing Buffer. Add 50 µl per injection).

   (If you are using the injector method you will need to order an additional amount of Nucleotide Releasing Buffer use Cat# ALX-K254-200-1)

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, 0.01, 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 minute 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.

IV. RELATED PRODUCTS:

- ApoSENSORTM ADP/ATP Ratio Assay Kit
- Apoptosis Detection Kits & Reagents
- Cell Fractionation System
- Cell Proliferation & Senescence
- Cell Damage & Repair
- Signal Transduction
- Adipocyte & Lipid Transfer
- CETP and PLTP Activity Assay Kits
- Cholesterol Assay Kit
- Molecular Biology & Reporter Assays
- Growth Factors and Cytokines
- Quality Antibodies for Apoptosis and Signal Transduction Molecules

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of a different assay buffer	• Refer datasheet and proceed accordingly
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Lysates used after multiple free-thaw cycles	• Aliquot and freeze sample lysates, if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		