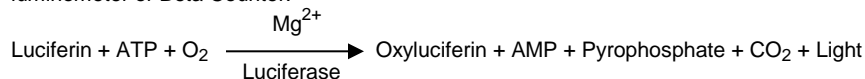


ApoSENSOR™ Cell Viability Assay Kit

(Catalog # K254-200, -1000; 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 Assay Kit** utilizes the 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 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	K254-200	K254-1000	Part Number
	200 assays	1000 assays	
Nucleotide Releasing Buffer	20 ml	100 ml	K254-xxx(x)-1
ATP Monitoring Enzyme	1 vial	5 vials	K254-xxx(x)-2
Enzyme Reconstitution Buffer	2 ml	5 x 2 ml	K254-xxx(x)-3
ATP	1 mg	1 mg	K254-xxx(x)-4

III. ApoSENSOR™ Cell Viability Assay Protocol:

A. Reagent Reconstitution and General Consideration:

- Reconstitute ATP Monitoring Enzyme with 2 ml/vial of the Enzyme Reconstitution Buffer. Mix well by gentle pipetting. The reconstituted enzyme is stable for up to 2 months at 4°C.
- Protect the ATP Monitoring Enzyme from light.
- Prepare an ATP standard solution by dissolving the 1 mg ATP into 1 ml of H₂O. 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 10³ – 10⁴ 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 (RT) 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).

FOR RESEARCH USE ONLY! Not to be used on humans.

B. Sample Assay Protocol:

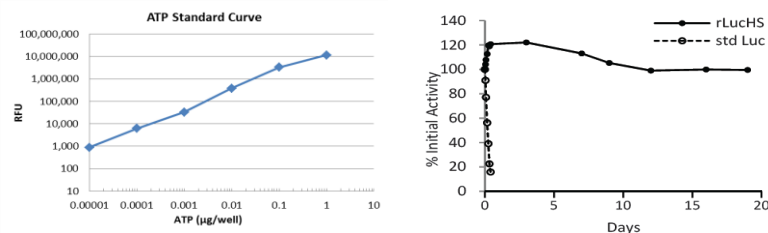
1. Induce apoptosis in cells by any desired method. Concurrently incubate a control culture without treatment.
2. For suspension cells, transfer 10 µl of the cultured cells (containing 10³ – 10⁴ cells) into luminometer plate. Add 100 µl of the Nucleotide Releasing Buffer.
For adherent cells, remove culture medium and treat cells (10³ – 10⁴) with 100 µl of Nucleotide Releasing Buffer for 5 min at RT with gentle shaking.
3. Add 10 µl ATP Monitoring Enzyme to the cell lysate. Read the sample within ~ 1-2 min 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 Nucleotide 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# K254-xxx(x)-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 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 µg/µl, etc) to generate serial ATP Standard at 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001 µg/well. Also include a 0 µg/well Sample to measure the Background luminescence) to luminometer plates. Then add 100 µl of Nucleotide Releasing Buffer and 10 µl of ATP Monitoring Enzyme. Read the Samples in 1 min 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.



Figures: (a) ATP Standard Curve. **(b)** Stability of luciferase at RT from *Diaphanes pectinealis* (rLucHS) as compared to Standard luciferase from *Photinus pyralis*.

RELATED PRODUCTS:

- ApoSENSOR™ ADP/ATP Ratio Assay Kit
- Apoptosis Detection Kits & Reagents
- Cell Fractionation System
- Cell Proliferation & Senescence

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of a different assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Refer datasheet and proceed accordingly • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Lysates used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze sample lysates, if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
<p>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		

GENERAL TROUBLESHOOTING GUIDE:

