ab65314

Cell Viability Assay Kit (Bioluminescent)

Instructions for Use

For the rapid, sensitive and accurate detection of apoptosis and cell proliferation in various samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

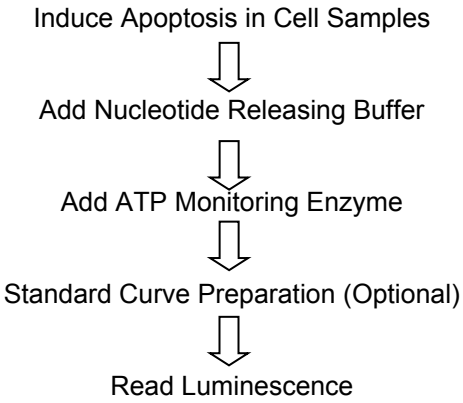
Cell death (especially apoptosis) is an energy-dependent process that requires ATP. As ATP levels fall to a point where the cell can no longer perform basic metabolic functions, the cell will die. A typical apoptotic cell exhibits a significant decrease in ATP level. Therefore, loss of ATP level in cells has been used as an indicator of cell death. In contrast, cell proliferation has been recognized by increased levels of ATP.

Abcam's Cell Viability 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.

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

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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Nucleotide Releasing Buffer	20 mL
ATP Monitoring Enzyme (Lyophilized)	1 vial
Enzyme Reconstitution Buffer	2 mL
ATP (MW 551)	1 mg

* Store kit at -20°C. 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.

ATP MONITORING ENZYME: Reconstitute 2 mL/vial with Enzyme Reconstitution Buffer. Mix well by gentle pipetting. This produces a yellow-green milky-like solution (not clear solution). Protect from light as much as possible. Aliquot enough enzymes (10 µL per assay) for the number of assays to be performed then re-freeze the remainder at -20°C for future use. The reconstituted enzyme is stable for up to 6 months at -20°C.

ATP STANDARD SOLUTION: Dissolve the 1 mg ATP into 1 mL of H_2O. The solution is stable for several weeks at -20°C.

Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Luminometer plate or Beta Counter
- 96-well plate
- Cuvettes
- Orbital shaker

4. Assay Protocol

Note: The Cell Viability Assay 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.

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).

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

2. **Sample Preparation:**
   a. **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.

   b. **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 within approximately 1 to 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 un-induced control.

Notes:

The assay can be analyzed using cuvette-based luminometers or Beta Counters. When a Beta Counter is used it should be programmed in the “out of coincidence” (or Luminescence mode) for measurement.

a) The entire assay can be done directly in a 96-well plate.

b) The assay 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.

5. Standard Curve (Optional):

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 for 1 minute in a luminometer (as described above). The background luminescence should be subtracted from all readings. The amount of ATP in un-induced and induced experimental samples can then be calculated from the standard curve.

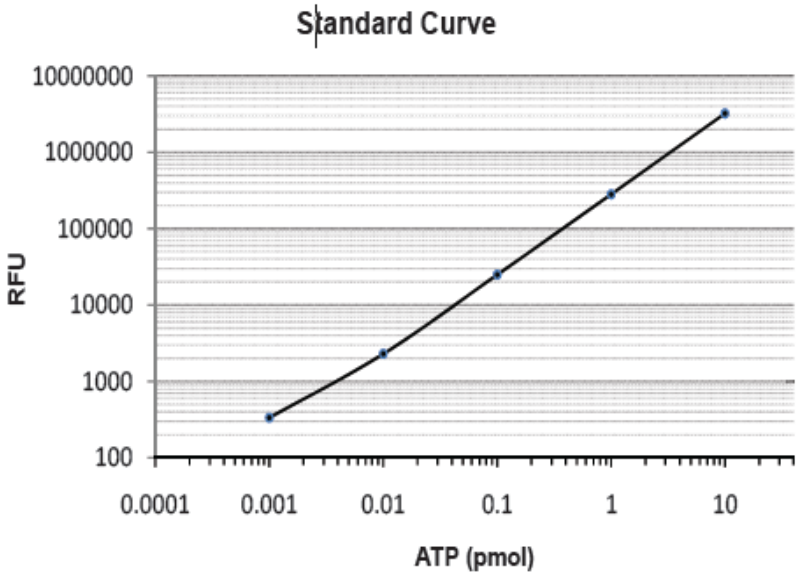


Figure 1: ATP Standard Curve.

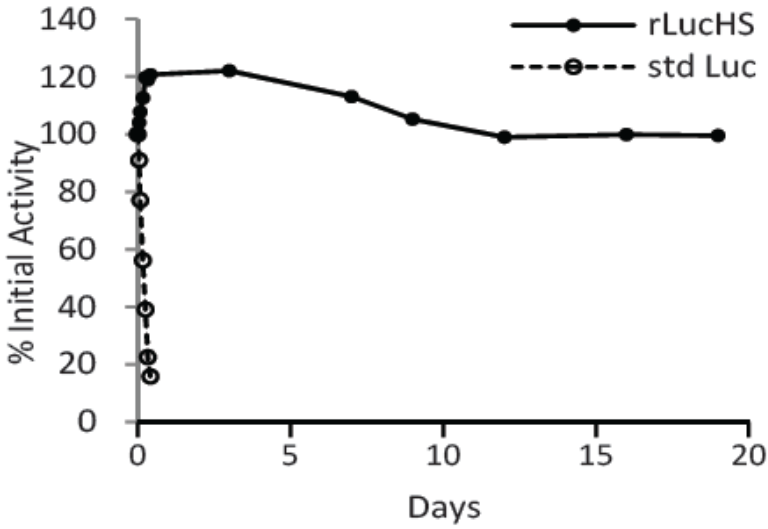


Figure 2: Stability of luciferase at room temperature from *Diaphanes pectinealis* (rLucHS) as compared to standard luciferase from *Photinus pyralis*.

5. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of a different assay buffer	Refer datasheet and proceed accordingly
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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