Rev. 0

ScienCell™ Research Laboratories

ATP Cell Viability Luminescence Assay (ATP-CVL)

Catalog # 9078

100 tests

Product Description

Luminescence intensity is directly proportional to intracellular ATP (adenosine triphosphate), enabling a sensitive and reliable measurement of cell viability across multiple cell types. Because ATP is present only in metabolically active cells and rapidly declines during cell death, the luminescent output reflects the number of living cells in culture. The ATP Cell Viability Luminescence Assay Kit (ATP-CVL) provides a simple add-mix-read workflow that requires no washing, harvesting, or cell transfer. The assay produces a strong luminescent signal suitable for high-throughput screening, drug cytotoxicity studies, cell proliferation analysis, and investigations of cellular metabolism in mammalian cells. [1–2].

Kit Components

Cat. #	# of vials	Product Name	Quantity	Storage
9078a	1	Cell Lysis & Cofactor Buffer (5X)	1.5 mL	4°C
9078b	1	Enzyme Detection Reagent	1 mL	-80°C (protect from light)
9078c	1	Enzyme Component	2 mg	-20°C (protect from light)
9078d	1	ATP Standard Solution	1 mL	-20°C (protect from light)
GQ100-5	1	Nuclease-free H2O	5 mL	4°C

Additional Materials Required (Materials Not Included in Kit)

Product Name
Mammalian cells in culture
Appropriate cell culture medium
Nutlin-3a or other experimental compounds
Dulbecco's Phosphate-Buffered Saline (DPBS, Cat#0303)
White or opaque 96-well plate
Luminometer or plate reader capable of luminescence detection

Product Use

ATP-CVL is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Quality Control

Assay performance was validated in HEK293T cells treated with Nutlin-3a in a 96-well plate format. Luminescence signal showed strong linearity with ATP standards ($R^2 > 0.99$) and robust signal-to-background ratios, confirming assay sensitivity and dynamic range (Figure 1 and 2).

Shipping and Storage

Kit components are shipped on dry ice. Upon receipt, store the product according to the conditions specified in the kit components table above.

References

[1] Gilbert D, et al. (2011). A Novel Multiplex Cell Viability Assay for High-Throughput RNAi Screening. *PLoS One*. Dec 5;6(12).
[2] Crouch SPM, et al. (1993). Comparison of MTT and ATP-based assays for the measurement of viable cell number *Journal of Bioluminescence and Chemiluminescence.*

Procedures

1. Reagent Preparation

A. Preparation of Cell Lysis & Cofactor Buffer 1X (6 mL total)

- Mix gently by inversion.:
  1.20 mL Cell Lysis & Cofactor Buffer 5X (Cat#9078a)
  4.80 mL Nuclease-free H2O (Cat#GQ100-5)

B. Preparation of ATP Working Reagent (6 mL total)

Important: Prepare fresh on the day of use, protect from light, and allow the reagent to reach room temperature before use.

- Add 1 mL Enzyme Detection Reagent (Cat#9078b).
- Add 5 mL Cell Lysis & Cofactor Buffer (1X).
- Add the entire vial of Enzyme Component (Cat#9078c).
- Mix gently by inversion 20–30 times. Do NOT vortex.
- Incubate on ice for 10 minutes to ensure complete dissolution.

C. ATP Standard Working Solution

Important: Prepare fresh on the day of use.

- Dilute the ATP stock solution (Cat#9078d) to generate a standard curve (e.g., 0, 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µM).

2. Procedure (Example Experiment: Nutlin-3a Cytotoxicity Assay)

A. Cell Seeding and Treatment

- Seed 10,000–30,000 cells per well in a white or opaque 96-well plate.
- Treat cells with Nutlin-3a at the desired concentrations (e.g., 1, 3, 5, 10 µM) in a final volume of 50–100 µL, in triplicate (n = 3).
- Include medium-only wells for background measurement.
- Incubate overnight at 37°C in a 5% CO_2 incubator.

3. ATP Standard Curve

A. Prepare ATP standards in assay buffer (0, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 µM final) and run in parallel wells.

B. Use the standard curve to interpolate sample RLUs and determine absolute ATP levels.

4. ATP Measurement

Important: Equilibrate plate and reagent to room temperature before starting.

A. Remove the plate from the incubator and equilibrate 5–10 minutes at RT.

B. Remove medium and gently wash cells with DPBS (Cat#0303).

C. Add 50 µL ATP Working Reagent to each well.
D. Incubate for 3 min at RT
E. Shake the plate for 10–30 seconds to mix.
F. Measure luminescence immediately.
Notes: Recommended luminometer settings: integration time 0.5–1 second per well. Signal remains stable for at least 10 minutes.

5. Data Analysis

- Subtracting background (lysis buffer only) from all RLUs.
- % Viability = 100 × (RLU_sample / RLU_control).
- % Cytotoxicity = 100 − % Viability.

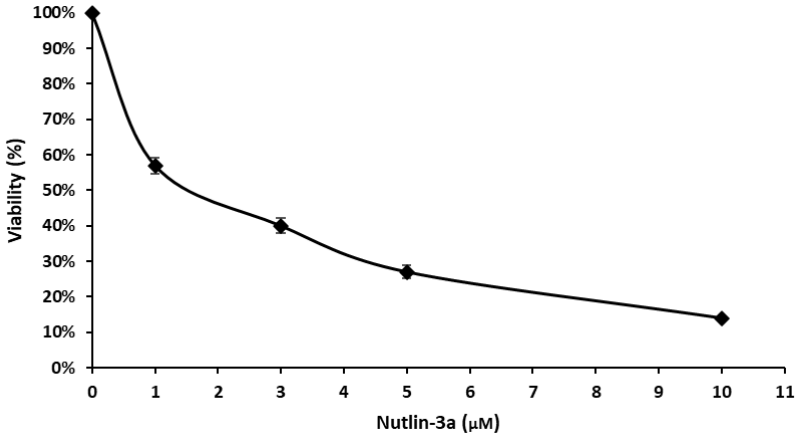

Figure 1. Dose-dependent reduction in HEK293T cell viability following 1–10 µM Nutlin-3a treatment measured by ATP-CVL. Luminescence (ATP) was normalized to untreated control (100% viability). Data represent mean ± SD (n = 3).

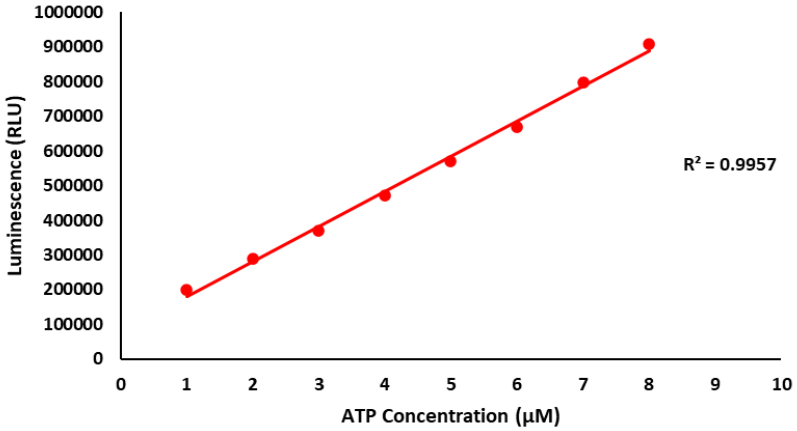

Figure 2. ATP standard curve showing a linear relationship between ATP concentration (0–10 µM) and luminescence (RLU) using the ATP-CVL assay, demonstrating high sensitivity and a broad dynamic range for ATP quantification in mammalian cells.