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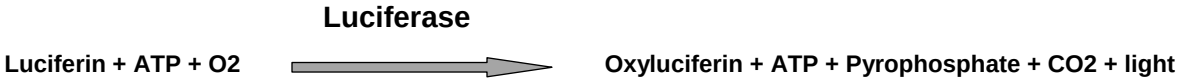
Elite™ Cell Growth (ATP) Assay Kit (Luminescence)

CATALOG NUMBER: CA-A115, 100 assays

Description

Adenosine triphosphate (ATP) plays a fundamental role in cellular energenics, metabolic regulation and cellular signaling. The quantitation of ATP can be used for a variety of biological applications. Because ATP is the energy source for almost all living organisms that rapidly degrades in the absence of viable organisms, its existence can be used to identify the presence of viable organisms. The measurement of ATP has been used for cell cytotoxicity, detection of bacteria on surfaces, quantification of bacteria in water, somatic cells in culture and food quality.

The Elite™ Cell Assay Kit is designed to gauge cell growth by measuring proportional changes in total cell ATP. This is a homogenous assay, which can be performed on multi-well plates by adding the reagents directly to cell growth medium in both adherent and suspension cell cultures. This makes it ideal for automated high-throughput screening. This assay has been optimized to accurately measure cell number over a broad dynamic range (10^2 - $2x10^5$ cells) in 96-well plate format. The assay accurately monitors cell growth over time periods from 24 – 120 h, and the ATP detection sensitivity is from 0.1 to 1000 µM.



Features

- **Continuous:** Homogenous assay, add-and-read assay, amenable to HTS format.
- **Wide Linear Range:** The assay generates a linear signal while measuring 100 to 200,000 cells. It is particularly suitable for determining the proliferation of fast-growing cancer cells in typical 3-5 day growth assays.
- **Sensitive and Accurate:** As low as 100 cells can be accurately quantified, and the growth curves correlate with those generated by traditional cell number counts.
- **Robust:** Excellent signal to noise (basal) ratio. Stable assay signal.

Applications

- Cell proliferation
- Cell toxicity
- ATP measurement

Kit Contents

Size	100 Assays (1x 96-well plate)	1000 Assays (10x 96-well plates)
Assay Solution I	5 ml	50 ml
Assay Solution II	5 ml	50 ml
Component A	50 µl	500 µl
Component B	30 µl	300 µl

Storage

Keep in freezer (-20 °C) and avoid exposure to light.

Materials Required (not supplied)

- 96 microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Microplate luminometer.

Assay Protocol

Preparation of Working Reagent

1. Warm the Assay Solution I and II at 37 ºC until completely thawed. Prepare **1X Intermediate Solution** by mixing Solution I and II at 1:1 ratio. Keep **1X Intermediate Solution** at room temperature (~22 ºC). Assay Solution I and II can be repeatedly frozen and thawed without loss of activity. **1X Intermediate Solution** should be prepared freshly every time.
2. Thaw components A & B at room temperature. Keep Components A and B on ice. ***Note: Do not thaw Component A & B at 37 ˚C.***
3. Transfer 50 µl of Component A and 30 µl of Component B into 10 ml of **1X Intermediate Solution** to make **Working Reagent**. The amount of **Working Reagent** prepared can be modified to suit experimental design. Unused Components A, B, and Assay Solution I and II can be refrozen (at -20 ˚C) for future experiments.
4. Mix thoroughly by inversion and vortexing for 30 sec. The reconstituted **Working Reagent** at room temperature should be used within 1 h, or it can be kept at 4 ºC for no more than 4 h. In general, only the freshly prepared **Working Reagent** is recommended for the experimental work.

Measurement of ATP in Growing Cells

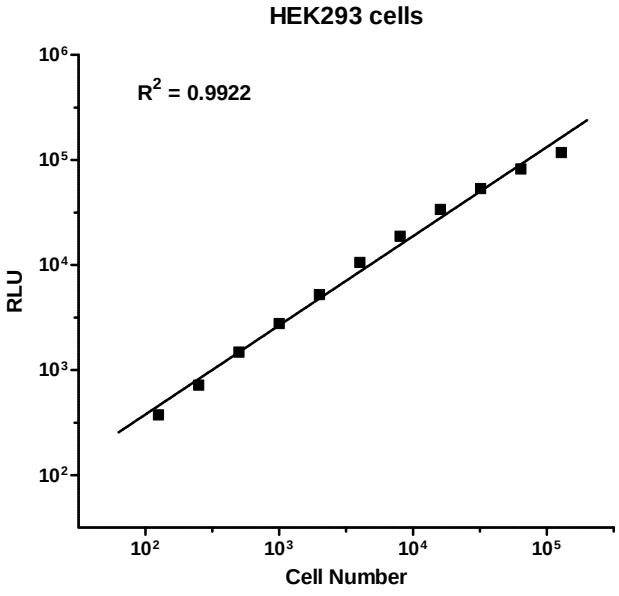
1. Grow cells on opaque white 96 well plates appropriate for luciferase signal measurement.
2. To generate assay signal, add an equal volume of **Working Reagent** to culture media overlaying cells.
3. Incubate the plate at room temperature (~22 ºC) for 20 min.
4. Read in a luminescence plate reader, record the data.

Generating an ATP standard curve

1. Dilute ATP in 1X HBSS. Transfer a fixed amount of ATP solution into the wells of a microtiter plate (e.g.,80 µl for a 96-well plate, and 20 µl for a 384-well plate).
2. Add an equal volume of **Working Reagent** into each well. Incubate the plate at room temperature (~22 ºC) for 20 min.
3. Read in a luminescence plate reader and record the data.

Data Analysis

A) Cell Dilution Curve



B) ATP Standard Curve

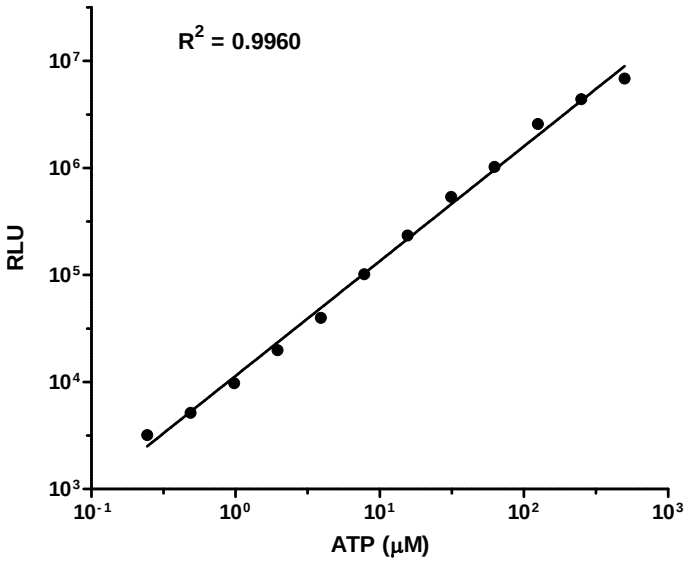


Figure 1. A) Different numbers of HEK293 cells were plated in a 96-well plate. **Working Reagent** (equal to cell growth media volume) was added into each well. Data was recorded 15 min later. **B)** A 1:2 serial dilution of ATP was prepared in HBSS. ATP solution (50 µl) was transferred into each well of a 96-well plate. The same volume of **Working Reagent** was added to each well. Data was recorded 15 min later.