## Overview

**Product name**  
Luminescent ATP Detection Assay Kit

**Detection method**  
Luminescent

**Sample type**  
Adherent cells, Suspension cells

**Assay type**  
Quantitative

**Assay time**  
0h 30m

**Product overview**  
Luminescent ATP Detection Assay Kit (ab113849) is used to measure the level of ATP within the cell. The luminescent ATP assay protocol involves lysis of the cell sample, addition of luciferase enzyme and luciferin, and measurement of the emitted light using a tube or microplate-based luminometer.

This kit irreversibly inactivates ATP degrading enzymes (ATPases) during the lysis step, ensuring that the luminescent signal obtained truly corresponds to the endogenous levels of ATP.

Luminescent ATP assay protocol summary:
- add ATP standard into standard wells and media into control wells in same plate containing cells to be analyzed
- add detergent solution and incubate for 5 min to lyse cells and stabilize ATP
- add substrate solution and incubate for 5 min
- store plate in dark for 10 min
- analyze on luminescence plate reader

**Notes**  
Total levels of cellular ATP can be used to assess cell viability, cell proliferation and cytotoxicity of a wide range of compounds and biological response modifiers.

We also offer a very popular alternative colorimetric/fluorometric ATP assay kit ab83355 based on the phosphorylation of glycerol.

**Related assays**

Review the cell health assay guide to learn about kits to perform a cell viability assay, cytotoxicity assay and cell proliferation assay.

Review the metabolism assay guide to learn about assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also about how to assay metabolic function in live cells using your plate reader.

**Platform**  
Microplate reader
**Storage instructions**

Store at +4°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergent</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>Lyophilized ATP standard</td>
<td>1 vial</td>
</tr>
<tr>
<td>Lyophilized substrate</td>
<td>3 vials</td>
</tr>
<tr>
<td>Substrate Buffer</td>
<td>1 x 20ml</td>
</tr>
</tbody>
</table>

**Properties**

**Components**

- **300 tests**

- **Detergent**: 1 x 20ml
- **Lyophilized ATP standard**: 1 vial
- **Lyophilized substrate**: 3 vials
- **Substrate Buffer**: 1 x 20ml

**Total cellular ATP concentration.** ATP in SH-SY5Y cells cultivated at 21% and 5% O2 24 h after treatment with A? peptide and/or 18 h X-ray irradiation, normalized to cell count, and compared to respective controls. ATP concentration was about 1.3- to 1.8-fold higher at all conditions in cells cultivated at 5% O2 compared to 21% O2. Combination of A? peptide treatment and irradiation resulted in a significantly increased (~1.5-fold) ATP concentration at 5% O2 compared to the control. Samples were measured at least in duplicates (n = 2-4) in three independent experiments (N = 3). Mean ± SEM analyzed by two-way ANOVA with Tukey's multiple comparison test with p < 0.05 considered as significant. (p < 0.01).

**Example of ATP standard curve using an opaque white plate**

The ATP standard curve was prepared as described in the protocol. Background-subtracted data values (mean +/- SD) are graphed.

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**Functional Studies - Luminescent ATP Detection Assay Kit (ab113849)**

Džinić, Tamara and Norbert A Dencher. Oxidative medicine and cellular longevity vol. 2018 7567959, Fig 6, doi:10.1155/2018/7567959
ab113849 ATP detection kit cytotoxicity data. 25000 HepG2 cells were seeded into each well, allowed to adhere and treated for 4 hours with 25µM rotenone and vehicle control (DMSO) in glucose based complete media. After treatment, cells were lysed, exposed to the ATP substrate solution and signal was measured on a luminescent counter. Mean and standard deviation is plotted for 3 replicates from each condition. Rotenone induces cytotoxicity in HepG2 cells.

Cellular Energy Flux for HepG2 cells (seeded at 65,000 per well), treated with a combination of drug compounds modulating the ETC (Antimycin A [1 µM] and FCCP [2.5 µM]), shown as a percentage relative to untreated control cells. Comparative measurements were taken with Extracellular Oxygen Consumption Assay (ab197243) (white column) and Glycolysis Assay [Extracellular acidification] (ab197244) (black column) show the shift between mitochondrial respiration and glycolysis and the cellular control of energy (ATP; measured 1h post-treatment using Luminescent ATP Detection Assay kit (ab113849) (striped column)).

Analysis of the release of ATP by connexin hemichannels in stem cells using ATP luminescence kit (ab113849).

Cells were cultured in HBSS to induce hemichannel opening. Calcium and GAP-inhibitor were used to trigger hemichannel closure.

After two hours the supernatant was collected and ATP was measured according to the protocol (detergent was also applied). Calcium treatment and inhibition by GAP decreased ATP concentration, compared to HBSS control. Graph shows data of three independent experiments.

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