ATP Determination Kit

Product Information

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Cat. number</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP Determination Kit</td>
<td>200-1000 assays (10 ml)</td>
<td>FP-S2841A</td>
</tr>
<tr>
<td>ATP Determination Kit</td>
<td>600-3000 assays (30 ml)</td>
<td>FP-S2841B</td>
</tr>
<tr>
<td>ATP Determination Kit</td>
<td>2000-10000 assays (100 ml)</td>
<td>FP-S2841C</td>
</tr>
</tbody>
</table>

Each kit contains:
- Component A: Firefly Luciferase (ready to use glycerol stock solution)
- Component B: D-Luciferin (solid, to dissolve in reaction buffer)
- Component C: Dithiothreitol (DTT) (solid, to dissolve in reaction buffer)
- Component D: Reaction Buffer (ready to use buffer, that can also be used for cell lysis)

Storage: Upon receipt all components should be stored 4°C (or at –20°C for long-term) (K). Follow the instructions below for the final reagent preparation.

Introduction

The ATP Determination Kit, sensitive assay, offers a convenient bioluminescence assay for quantitative determination of small amounts of ATP. Catalysed by firefly luciferase the substrate D-luciferin is oxidized in an ATP-dependent process generating chemiluminescence at 560 nm (pH 7.8):

\[
\text{luciferin} + ATP + O_2 + Mg^{2+}, \text{luciferase} \rightarrow \text{oxyluciferin} + ATP + pyrophosphate + CO_2 + \text{light}
\]

The sensitive assay is optimized for fast determination of low levels of pre-existing ATP or ATP formed in kinetic systems. After a 10 min incubation of the assay reagent, ATP concentrations down to 0.1 pmol can be exactly determined using the linear luminescent signal of the luciferase reaction. Loss of luminescent signal and sensitivity is observed after incubation times of more than 30 minutes. If you are interested in a time-stable assay (i.e. for high throughput screenings) with nearly constant luminescence signals over a period of up to four hours, use our time-stable ATP Determination Kit.

Linear luminescence signal for ATP concentrations down to 0.1 pmol using the ATP Determination Kit, sensitive assay
**FluoProbes®**

**Direction for use**

**Handling and Storage – Reagent preparation**

Table for working stock solution and final assay reagent:

<table>
<thead>
<tr>
<th>Preparation of working/stock solutions</th>
<th>10ml kit</th>
<th>30ml kit</th>
<th>100ml kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Luciferin: to vial (B), add reaction buffer (D) volume:</td>
<td>+500µl of D</td>
<td>+500µl of D</td>
<td>+500µl of D</td>
</tr>
<tr>
<td>DTT: to vial (C), add reaction buffer (D) volume:</td>
<td>+150µl of D</td>
<td>+500µl of D</td>
<td>+1000µl of D</td>
</tr>
</tbody>
</table>

**Preparation of final Assay Reagent Mix**

<table>
<thead>
<tr>
<th>Reaction Buffer (D)</th>
<th>DTT stock solution</th>
<th>Luciferin working solution</th>
<th>Luciferase (A), ready to use glycerol stock solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>9755 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>45 µl</td>
</tr>
<tr>
<td>29.37 ml</td>
<td>300 µl</td>
<td>300 µl</td>
<td>30 µl</td>
</tr>
<tr>
<td>98.5 ml</td>
<td>1 ml</td>
<td>400 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

**Storage:**

Upon receipt store all kit components at –20°C. Please equilibrate the component B, C, D to room temperature and Component A on ice before use and follow the instructions for the final reagent preparation. The finished reagent should be used immediately or dispensed into aliquots and stored at –20°C (stable for at least 6 months).

Avoid repeated freezing and thawing. Protect from light.

**Comments:**

- **D-Luciferin stock solution:** Add Reaction Buffer (Component D) to the D-Luciferin (Component B) according above table and mix gently to dissolve the D-Luciferin completely. This D-Luciferin stock solution should be protected from light and is reasonable stable for several days at 4°C.

- **DTT stock solution:** Add Dithiothreitol (Component C) according above table and dissolve it completely.

- **The final reagent mix** is prepared according above table with Reaction Buffer (Component D), DTT stock solution, D-Luciferin stock solution and Firefly Luciferase (Component A, only small volume, please centrifuge shortly if complete volume is not at the bottom of the vial). Mix solutions containing luciferase gently by inversion – vortex mixing may denature the enzyme.

**Protocol**

1- Thaw aliquot of final reagent mix and allow it to reach room temperature.

2- Add equal volume of final reagent reagent mix and of ATP solution (sample, standard) to be determined in a white 96 or 384 well plate optimised for luminescent reading. Suggested volumes:

<table>
<thead>
<tr>
<th>Assay Reagent Mix</th>
<th>10ml kit</th>
<th>30ml kit</th>
<th>100ml kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Reagent Mix</td>
<td>50µl</td>
<td>50µl</td>
<td>50µl</td>
</tr>
<tr>
<td>ATP standard or sample</td>
<td>50µl</td>
<td>50µl</td>
<td>50µl</td>
</tr>
</tbody>
</table>

**NB:** Different assay formats are possible, but take care that equal volumes of reagent and ATP solution are mixed.

3- The luminescent signal can be measured preferably in a luminometer after 10 minutes at room temperature. Do not expand incubation times longer than 30 minutes before reading luminescence because signal sensitivity is lost during longer incubations and calibration curves become inaccurate. Background luminescence can be subtracted if a blank assay was performed with buffer or pure water instead of an ATP containing solution.

4- Alternatively to a luminometer a scintillation counter can be used to measure luciferase activity, too. Make a significant dilution (in 1x Reaction Buffer supplemented with 1mg/ml BSA) of the sample in a clear or translucent vial so that the sample completely covers the bottom of the vial (the sample can also be placed in a microfuge tube in the vial). Do not add scintillant! For a linear relationship between luciferase concentration and counts per minute (cpm), the coincidence circuit on the scintillation counter should be turned off. If it can not be turned off, you have to calculate the square root of measured cpm minus background cpm using a water or buffer blank ([sample-background]^{1/2}). The scintillation counter must be used in manual mode and should be read individually for 1-5 minutes each.

5- To determine the ATP concentrations, it is necessary to generate a standard curve for a series of defined ATP concentrations (range of 10^{-16} to 10^{-6} moles of ATP, i.e. 10^4 to 10^7 M). For the determination of unknown ATP concentrations use reproducible experimental conditions (temperature, incubation times, assay volume, luminometer adjustments, etc.). Highest reproducibility of data is achieved, when luminescence is directly read after a fixed incubation time of 10 minutes.

6- The sensitive ATP Determination Kit is optimised for a total content of 0.1 to 100 pmol ATP per assay with a linear fit of the standard curve. Dilute higher ATP concentrations to obtain best results. For a time stable ATP assay with a nearly constant luminescence signals up to 4 hours order our ATP Determination Kit, Time stable assay order-no. BU1200 for 10ml or order-no BU1202 for 100 ml

Note that the time stable assay is optimized for ATP concentrations of 10 nM to 10 µM!
FluoProbes®

FT-S2841A

Cell preparation - Cell lysis buffers

Many human cells can be assayed directly using our kit. However, strong cells may require a cell lysis step. Cell lysis can be performed by a freeze-thaw cycle, but that will not destroy ATPases completely, so even when the time stable assay is used, the ATP luciferase signal will decrease within one hour.

More reproducible results are achieved when samples are spun down carefully, resolved in Component D buffer of our kits as lysis buffer and heated for 5 min at 95 degrees - this will lyse cells and block ATPases! If you are interested in this method, please order a bigger volume of Component D (FP-CA3920, 30 ml).

Adhesive cells first have to be removed from the surface by conventional ways, spun down washed with buffer and lysed by known techniques or simply heated for 5 min in our Component D reaction buffer.

Some Bacteria can be lysed in our reaction buffer in 5 min shaking at 95 degrees. However, if you intend to assay bacteria or plant cells you have to use your own protocol for cell lysis.

Cell lysis buffers may be prepared, containing:
- ATPase inhibitors as DTAB (0.05 - 2%), BDDABr, sulfobetaine 3-10, SDS*, deoxycholate* and NaF (1-20mM)
- Concentrations only up to 0.05%
- Cell-lysing agents like TCA, DMSA, CTAB or ethanol
- Enzyme stabilising agents like THESIT (1-5%), BSA or gelatin.

References

- Tissier R. et al., Rapid cooling preserves the ischaemic myocardium against mitochondrial damage and left ventricular dysfunction, Cardiovascular Research, 83(2):345-353 (2009) Abstract

Related products

- ATP Determination kit, 4h reading, FP-BU1200
- ATP disodium salt, MW 605.2, 00064A
- Component D of the ATP determination kit, FP-CA3920
- HydroSafe™-I for disinfecting water baths of C02 incubators, CB2840
- H2DCFDA, FP-467312
- FPLyte 96 Wells Plate, White Body & Bottom, CP6331

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : Fluoprobes® / Interchim; Hotline : +33(0)4 70 03 73 06

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