Biaffin GmbH & Co KG
Reagents, chemicals and proteins for
exploring cellular signalling networks

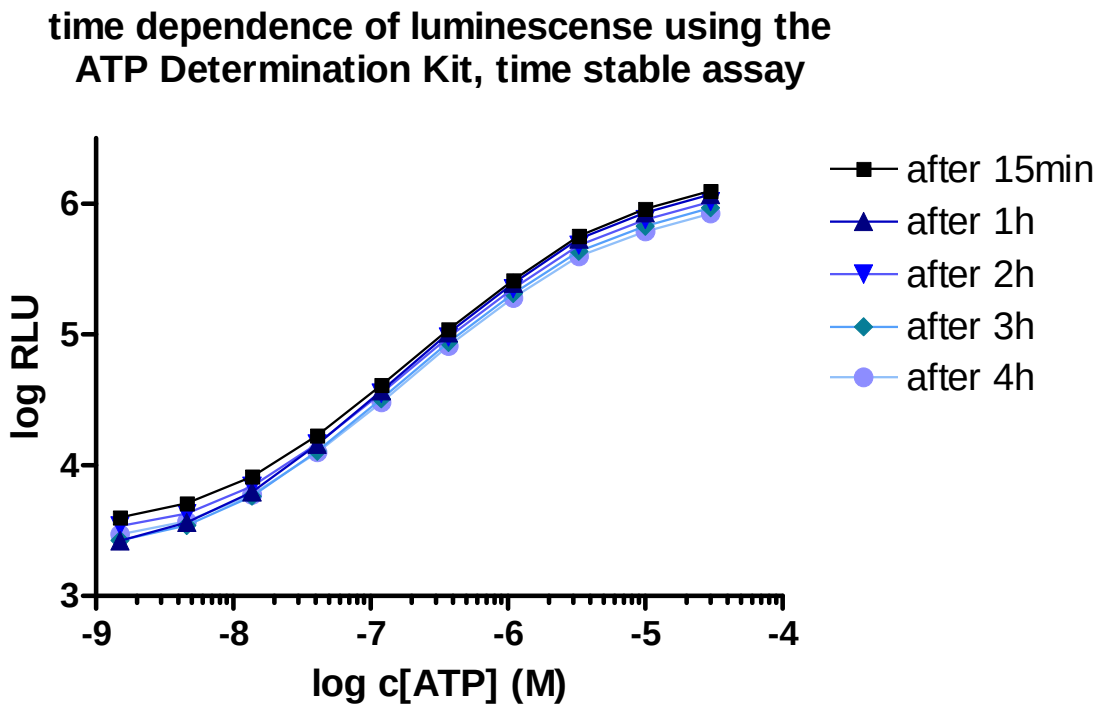
ATP Determination Kit, time stable assay

1. Description

The ATP Determination Kit, time stable assay, offers a convenient bioluminescense 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):

$$luciferin + ATP + O_2 \xrightarrow{Mg^{2+},luciferase} oxyluciferin + AMP + pyrophosphate + CO_2 + light$$

The timestable assay is optimized for high throughput screening with nearly constant luminescence signals over a period of up to four hours. The sensitive assay can detect nanomolar to micromolar concentrations of pre-existing ATP or ATP formed in kinetic systems.



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2. Materials

Product:

ATP determination Kit, time stable, 10 ml for 200 – 1000 assays: order-no. LBR-T010

Kit contents *(see Safety Material Data Sheets for handling)*:

- Firefly Luciferase (Component A, ready to use glycerol stock solution)
- D-Luciferin (Component B, solid, to dissolve in reaction buffer)
- Dithiothreitol DTT (Component C, solid, to dissolve in reaction buffer)
- Reaction Buffer (Component D)

Storage:

Upon receipt all components should be stored 4°C. Follow the instructions for the final reagent preparation.

3. Experimental Protocol:

Reagent Preparation

- D-Luciferin stock solution: Add 500 µl of Reaction Buffer (Component D) to the D-Luciferin (Component B) 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 150 µl of Reaction Buffer (Component D) to Dithiothreitol (Component C) and dissolve it completely.
- The final reagent mix is prepared of 9850 µl Reaction Buffer (Component D), 100 µl DTT stock solution, 40 µl D-Luciferin stock solution and 10 µl 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.

  (Note: you can improve the resulting luminescent signal by increasing the D-Luciferin concentration in the reagent by adding up to 400 µl of the D-Luciferin stock solution in 10 ml reagent, but this will cause a strong loss of signal stability during time, so time stable measurements can only be performed in less than one hour!)
- Make suitable aliquots and store them light protected until use at –20°C. Avoid repeated freezing and thawing.

Standard Reaction

- Thaw aliquot of final reagent mix and allow it to reach room temperature.
- For 200 assay format add 50 µl of final reagent mix to 50 µl of ATP solution to be determined in a white 96 or 384 well plate optimised for luminescent reading; for 1000 assay format mix 10 µl of final reagent mix with 10 µl ATP solution. Other assay formats are possible, but take care that equal volumes of reagent and ATP solution are mixed.
- The time stable luminescent signal can be measured preferably in a luminometer after 10 minutes to 4 hours at room temperature. Background luminescence can be subtracted if a blank assay was performed with buffer or pure water instead of an ATP containing solution.
- 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 ($[\text{sample-background}]^{1/2}$). The scintillation counter must be used in manual mode and should be read individually for 1-5 minutes each.
- To determine the ATP concentrations, it is necessary to generate a standard curve for a series of defined ATP concentrations. For the determination of unknown ATP concentrations use reproducible experimental conditions (temperature, incubation times, assay volume, luminometer adjustments, etc.).
- The time stable ATP Determination Kit is optimised for ATP concentrations ranging from 10 nM to 10 µM with a linear fit of the standard curve. Dilute higher ATP concentrations to obtain best results. For lower ATP concentrations order our ATP Determination Kit, Sensitive Assay order-no. LBR-S010 for 10ml, order-no. LBR-S030 for 30ml or order-no. LBR-S100 for 100 ml.