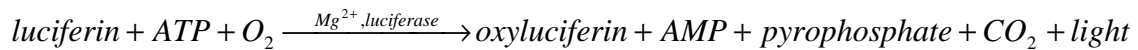


ATP Determination Kit, sensitive assay

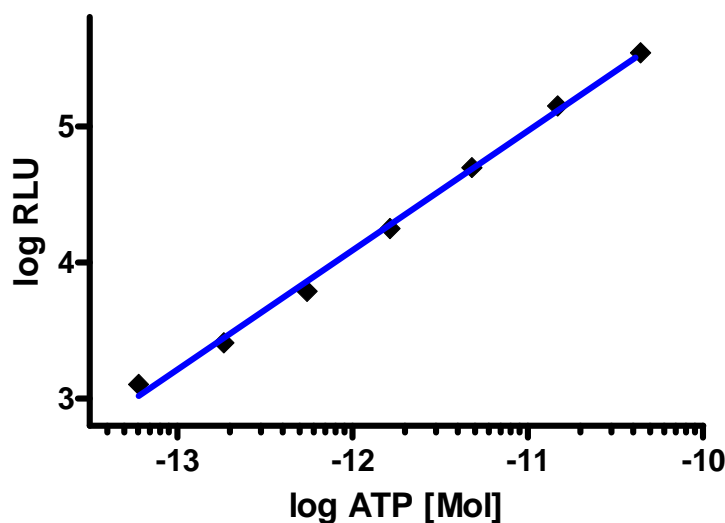
1. Description

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):



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 timestable assay (i.e. for high throughput screenings) with nearly constant luminescence signals over a period of up to four hours, use our timestable ATP Determination Kit.

linear luminescence signal for ATP concentrations
down to 0.1 pmol using the ATP Determination Kit,
sensitive assay



2. Materials

Product:

ATP determination Kit, sensitive, 10 ml for 200 – 1000 assays: order-no. LBR-S010

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, ready to use buffer)

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 9790 µl Reaction Buffer (Component D), 100 µl DTT stock solution, 100 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.
- 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 luminescent signal should be measured in a luminometer directly 10 minutes after sample preparation at room temperature. Do not extend incubation times longer than 30 minutes before reading luminescence because the signal sensitivity decreases rapidly with longer incubation times due to substrate depletion and calibration curves become inaccurate. Background luminescence can be subtracted using a blank sample with buffer or water instead of an ATP containing solution.
- As an alternative to a luminometer a scintillation counter can be used to measure luciferase activity. 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.). Highest reproducibility of data is achieved, when luminescence is directly read after a fixed incubation time of 10 minutes.
- 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. LBR-T010 for 10ml, order-no. LBR-T030 for 30ml or order-no. LBR-T100 for 100 ml (*Note that the time stable assay is optimized for ATP concentrations of 10 nM to 10 µM!*).