ATP Bioluminescence Assay Kit HS II

Reagent set for the highly sensitive and quantitative detection of ATP by luciferase driven bioluminescence

Cat. No. 11 699 709 001

1,000 assays (microwell plate format)
500 assays (tube format)

Version 11
Content version: June 2018
Store at −15 to −25°C

1. What this Product Does

Contents

Vial/Cap	Label	Contents/Function
1 white	Luciferase Reagent, lyophilized	5 × 10 ml
2 red	ATP standard, lyophilized	5 × 10 mg
3 blue	Cell Lysis Reagent, ready-to-use	• 100 ml • for lysis of eukaryotic and prokaryotic cells
4 green	Dilution Buffer, ready-to-use	• 100 ml • for dilution and reconstitution of Luciferase Reagent and ATP Standard

Storage and Stability

If stored at −15 to −25°C, the unopened kit is stable until the expiration date printed on the label.

The kit is shipped on dry ice.

Application

The ATP Bioluminescence Assay Kit HS II is used for highly sensitive and quantitative determination of ATP and can be applied for the detection of microbial contamination.

The ATP Bioluminescence Assay Kit HS II uses the ATP dependency of the light emitting luciferase catalyzed oxidation of luciferin for the measurement of extremely low concentrations of ATP (1). The ATP Bioluminescence Assay Kit HS II is optimized for highest sensitivity. The kit is especially suited for the use in tube-based as well as in microwell plate (MWP)-based luminometers. The preparation of an ATP calibration curve is facilitated using the stablized ATP standard stocks provided. The exact ATP content of the standard as indicated on the label, is determined individually for each lot by HPLC and spectroscopy. The kit contains a ready-to-use lysis reagent especially developed for the efficient lysis of a variety of eukaryotic and prokaryotic cells.

The ATP Bioluminescence Assay Kit HS II is especially developed for the detection of ATP with high sensitivity. Due to the high concentration of luciferase in the assay the reaction exhibits a peak kinetic (Fig. 1). If ATP determinations are performed to obtain kinetic data of enzymes involved (*e.g.,* for metabolic studies or if coupled enzymatic assays are applied), the use of the ATP Bioluminescence Assay Kit CLS II* (generating a constant light signal) is highly recommended.

** available from Roche Diagnostics*

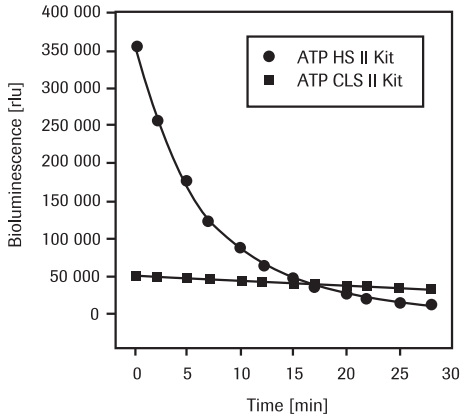

Fig. 1: Kinetics of light generation of the ATP Bioluminescence Assay Kit HS II and the ATP Bioluminescence Assay Kit CLS II. 10 pmol ATP in a volume of 50 µl was assayed with 50 µl luciferase reagent in a black microtiter plate.

Additional Equipment and Reagents Required

Additional equipment required to perform ATP determinations with the ATP Bioluminescence Assay Kit HS II include:

- tube- or MWP-based luminometer with suitable tubes or plates

Product Characteristics

Test Principle

The luciferase from *Photinus pyralis* (American firefly) catalyses the following reaction:

$$ATP + D\text{-luciferin} + O_2 \varnothing \text{ oxyluciferin} + PP_i + AMP + CO_2 + light$$

The quantum yield for this reaction is about 90%. The resulting green light has an emission maximum at 562 nm.

The Michaelis equation has the following form:

$$\text{light intensity} = (V_{max} \times C_{ATP})/(K_m + C_{ATP})$$

At low ATP concentrations ($C_{ATP} << K_m$) the formula simplified to light intensity $= V_{max} \times C_{ATP}/K_m$.

From this equation it becomes obvious that the light output is directly proportional to the ATP concentration (C_{ATP}) and dependent on the amount of luciferase (V_{max}) present in the assay. Therefore, for maximum sensitivity, the sample ATP has to be in a minimum volume and the luciferase reagent must not be diluted.

Working Range	The working range of the ATP Bioluminescence Assay Kit HS II is between 10^{-5} and 10^{-12} M ATP (Fig. 2).
Detection Limit	The detection limit for ATP, using a Berthold-type microplate luminometer LB-96-P, is in the range of 10^{-12} M (Fig. 2). Due to a high luciferase activity in the luciferase reagent, the ATP Bioluminescence Assay Kit HS II is optimized with respect to a more rapid turnover of the sample ATP. This leads to an enhanced light output and as a direct consequence to an improved sensitivity.

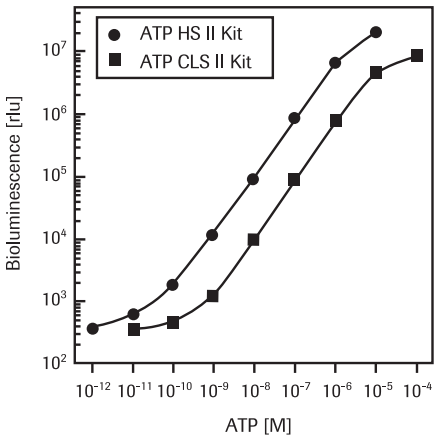

Fig. 2: Sensitivity range of the ATP Bioluminescence Assay Kit HS II and the ATP Bioluminescence Assay Kit CLS II. ATP dilutions in a volume of 50 µl were assayed with 50 µl luciferase reagent in a black microtiter plate on a Berthold LB-96-P luminometer. The light signal was integrated for 10 s after a delay of 1 s.

2. How to Use this Product

2.1 Before You Begin

Preparation of Working Solutions

Reagent	Preparation	Storage/Stability
Luciferase Reagent	Dissolve the whole content of one vial 1 by carefully adding 10 ml of dilution buffer from vial 4. Incubate for 5 min at 0 to +4°C without stirring or shaking. Mix for a homogeneous solution by carefully rotating the bottle. Do not shake!	• The reagent is stable for one day at +15 to +25°C or for one week when stored at 0 to +4°C. • However, set up a standard curve each day, because a slight loss of luciferase activity may occur during this time (approx. 20% after storage for 5 days at +2 to +8°C). • Reconstituted luciferase reagent may be stored frozen at −15 to −25°C for longer periods of time. ⚠ Each freeze/thaw-cycle reduces the luciferase activity to a certain degree, depending on the freezing conditions (shock freezing is most considerate). Therefore, avoid repeated freezing and thawing.
ATP Standard	Each bottle contains approx. 10 mg ATP (>98% purity; M_r 605.2). The exact amount of ATP is determined individually for each lot as indicated on the label. Dissolve the content of one vial 2 by addition of the appropriate volume of dilution buffer to get a final concentration of 10 mg/ml or 16.5 mM, respectively (e.g. 960 µl to 9.60 mg ATP). The ATP standard curve is prepared by serial dilutions of one ATP standard with dilution buffer.	• The ATP standard solution is stable for one week when stored at +2 to +8°C (<5% degradation). • When stored at −15 to −25°C, the solution is stable for at least 4 weeks (<5% degradation). • Diluted ATP-standards are stable for 8 hours when stored on ice.
Cell Lysis Reagent	The cell lysis reagent is ready-to-use and bottled under sterile conditions. ⚠ Avoid contamination by microorganisms or ATP. The use of autoclaved or heat sterilized labware is recommended.	The reagent is stable at +2 to +8°C.
Dilution Buffer	The dilution buffer supplied with the kit is essentially free of ATP and microbial contaminations. Use this buffer for reconstitution and dilution of the kit reagents. The residual buffer (44 ml) can be used as diluent for the samples. ⚠ Avoid contamination and use autoclaved or heat sterilized labware.	Store at +2 to +8°C.

Cell Lysis

Efficiency and reproducibility of the lysis procedure is a prerequisite for accurate ATP determinations in biological samples. Several methods are described to release ATP from somatic cells, bacteria, algae and yeast including organic solvents (ethanol, butanol), detergents or acids (TPA, PCA). Most of these extractants exhibit marked disadvantages like partial inhibition of the luciferase activity, or incomplete inactivation of endogenous ATP converting enzymes.

The lysis reagent included with the kit exhibits good lysis efficiency with respect to a variety of eucaryotic and procaryotic cells (Table 1) with hardly no influence on luciferase activity. However, some organisms (yeast, algae) are resistent at standard conditions. In these special cases the lysis reagent may also be tried at elevated temperature (up to 100°C) or with prolonged incubation times.

The boiling method is probably the most efficient and universal way to extract ATP from all the different kind of cells. The major disadvantage is, that this method is time consuming and inconvenient, especially, when larger sample numbers have to be processed. However, the boiling method is a useful reference method to prove the lysis efficiency of the lysis reagent with respect to altered conditions or special cells.

Cell type	Lysis efficiency
Microorganisms	
Bacillus cereus	101%
Staphylococcus aureus	91%
Klebsiella pneumoniae	99%
Pseudomonas aeroginosa	101%
Escherichia coli	108%
Candida albicans	4%
Scenedesmus obtusiusculus	2%
Somatic cells	
Erythrocytes	105%
Granulocytes	94%
Lymphocytes	108%
Platelets	96%
Fat cells	86%
Alveolar macrophages	33%
Lung fibroblasts	100%
Human placenta	54%

Tab. 1: Lysis efficiency of the lysis reagent compared to the boiling method (boiling method = 100%)

pH of the Sample

The luciferase reaction has its pH-optimum in the range of 7.75. Samples having extreme pH-values or samples of a different pH containing high buffer concentrations may influence the light reaction in an unpredictable way. To avoid problems, correct the pH to a value between 7.6 and 8.0 or dilute samples in tricine buffer of the appropriate pH. The buffer concentration in the HS-reagent is 25 mM.

Kinetic of the Light Reaction

Due to the high luciferase activity present in the assay mix, the ATP concentration of the sample decreases considerably with time. As a result, the light output declines fast from the start of the reaction as illustrated in Fig. 1.

In order to guarantee accuracy of the readings, the reagent volume, the injection conditions and the onset of signal integration has to be absolute reproducible. These parameters have to be considered, particularly, if the injections have to be done by hand.

Equipment

The reagent set can be used with all commercially available luminometers (tube-format or MWP-format). For microplate luminometers use white or black microtiter plates. Automated injection systems provide the option to add reagents directly in measurement position and to start light signal integration after a constant, selectable delay (4). This opens the possibility to process even a large number of samples in a convenient and highly reproducible way.

2.2 Procedure

The standard protocols as described below are general guidelines and first choice protocols but are open to variations upon special needs. Within certain limits the ratios of the assay components can be varied with having little influence on the sensitivity of the assay.

Determination of ATP

Step	Action	Volume	
		microwell plate	tube
❶	If necessary, dilute samples with dilution buffer (vial 4) to an appropriate ATP concentration.	50 µl sample	100 µl sample
❷	Dilute ATP standard with dilution buffer (vial 4) by serial dilution in the range of 10^{-10} and 10^{-16} M ATP (10^{-6} to 10^{-12} M).	50 µl standard	100 µl standard
❸	Add luciferase reagent to the samples/standards by automated injection and start measurement after a 1 s delay and integrate for 1 to 10 s.	50 µl reagent	100 µl reagent
❹	Subtract the blank from the raw data and calculate ATP concentrations from a log-log plot of the standard curve data.	variable	0.01 – 500 ng

Extraction of ATP from eukaryotic or prokaryotic cells (2,3)

Step	Action	Volume	
		microwell plate	tube
❶	• Dilute cells to a concentration of 10^5 to 10^8 cells/ml. • If the sample is too dilute, concentrate by centrifugation (bacteria and yeast: 8000 × *g*, 10 min; somatic cells: 900 × *g*, 10 min) and discard the supernatant carefully. Resuspend pellet in a minimal volume of dilution buffer (50–100 µl). • Concentration from large volumes can be performed by filtration through a 0.2 µm filter. The ATP can be released with cell lysis reagent directly from the filter (in a minimum volume). For the dilution of the samples 44 ml dilution buffer (vial 4) are available.	25 µl (minimum)	50 µl (minimum)
❷	Dilute ATP standard with dilution buffer (vial 4) by serial dilution in the range of 10^{-10} and 10^{-16} moles ATP (10^{-6} to 10^{-12} M).	25 µl (minimum)	50 µl (minimum)
❸	Add to sample or ATP standard the same volume of cell lysis reagent and incubate for 5 min at +15 to +25°C. Alternatively, add to the cell suspension 9 volumes of boiling 100 mM Tris, 4 mM EDTA, pH 7.75. Incubate for another 2 min at 100°C. Centrifuge sample at 10,000 × *g* for 60 s and transfer the supernatant to a fresh tube. Keep samples on ice until measurement. Centrifugation or other steps for cell debris separation is normally not necessary.		
❹	Transfer the appropriate volume of sample/standard prepared in step 2 into a MWP-well or tube.	50 µl	100 µl
❺	Add luciferase reagent to the samples/standards by automated injection and start measurement after a 1 s delay and integrate for 1 to 10 s.	50 µl reagent	100 µl reagent
❻	Subtract the blank from the raw data and calculate ATP concentrations from a log-log plot of the standard curve data.		

3. Troubleshooting

Problem	Possible Cause	Recommendation
No signal or signal too weak	Sample is too diluted.	Minimize volume prior to cell disruption, *e.g.* by filtration or centrifugation.
	ATP is destroyed by endogenous ATPases.	Minimize time between cell disintegration and inactivation (if a method other than boiling has to be used). Keep samples at 0 to +4°C. Test on ATPases by adding exogenous ATP to the sample.
	Excess of chelating agents in the sample capturing Mg^{2+} ions necessary for luciferase-activity.	Add an appropriate concentration of Mg^{2+} sufficient to compensate chelating agents. The luciferase reagent contains 10 mM Mg^{2+}.
	Luciferase reagent destroyed.	Dissolve a new vial of luciferase reagent. Do not stir or shake during dissolution. Store maximally for 5 days at +2 to +8°C, once dissolved. Avoid repeated freezing/thawing.
	pH of sample out of optimal range.	Adapt sample pH to 7.6–8.0.
	Measuring time too short.	Prolonge the integration time with your luminometer.
	Use of (wrong) filter in the luminometer.	Do not use any filters during measurement.
Non-linear calibration curve	Pipetting error	Pipette the serial dilutions carefully. Check precision of pipettes. Prepare the dilutions in triplicate.
	Cross talk between individual wells of the MWP.	Use only white or preferably black MWPs; do not use transparent MWPs.
	Measuring time for the total MWP is too long: substrate is depleted in lately measured standards.	Ensure that the time between addition of the luciferase reagent and measuring is as short as possible and constant for all samples. If time course measurements are desired, use ATP Bioluminescence Assay Kit CLS II*.

Problem	Possible Cause	Recommendation
Too high background	ATP contaminations in water, buffers, vials used.	Use all lysis reagent and dilution buffer supplied in the kit. Otherwise check cell lysis reagent and dilution buffer for ATP contaminations. Use only redist. water for the preparation of buffers used for cell harvesting and sample preparation. Check labware for ATP contaminations.
	Measuring time too long.	Decrease the integration time. Minimize volume prior to cell disruption e.g. by filtration or centrifugation.
Strong variations of ATP content between several identical experiments.	Cells are harvested using too harsh conditions.	Avoid high centrifugation speeds (for bacteria and yeast maximally 8,000 × *g*, for somatic cells maximally 900 × *g*). Reduce volume flow upon filtration of cells.
	No time-standardized cell harvesting conditions.	Standardize experimental time for all harvesting steps.
	Cross talk between individual wells of the MWP.	Use only white or preferably black MWPs; do not use transparent MWP's.
	If no automated MWP-luminometer is used: Measuring time for the total MWP is too long. Substrate or ATP is depleted in lately measured samples.	Ensure that the time between addition of the luciferase reagent and measuring is as short as possible and constant for all samples. If time course measurements are desired, use ATP Bioluminescence Assay Kit CLS II*.

4. Additional Information on this Product

Background Information

Living beings require a continual input of free energy for three major purposes: the performance of mechanical work in muscle contraction and other cellular movements, the active transport of molecules and ions, and the synthesis of macromolecules and other biomolecules from simple precursors.

The free energy used in these processes, which maintains an organism in a state that is far from equilibrium, is derived from the environment. In most processes, this special carrier of free energy is adenosine triphosphate (ATP). ATP is an energy-rich molecule because its triphosphate unit contains two phosphoanhydride bonds. The turnover of ATP is very high. Motion, active transport, signal amplification, and biosynthesis (as is needed for cell proliferation) can occur only if ATP is continuously regenerated from ADP. Therefore, measurement of ATP can serve as a marker for cell proliferation.

The determination of ATP using bioluminescence is a well established technique. It uses the ATP dependency of the light-emitting, luciferase-catalyzed oxidation of luciferin for the measurement of extremely low concentrations of ATP.

Quality Control

Quality control is performed with a 0.001 pmol and 1,000 pmol ATP standard. 0.001 picomol routinely yields at least 300-400 counts, 1,000 pmol 20-40 Mio counts.

References

1 De Luca, M. & McElroy, W.D. (1978) *Methods Enzymol.* **57**, 3–15.
2 Stanley, P.E. (1986) *Methods Enzymol.* **133**, 14–22.
3 Lundin, A. et al. (1986) *Methods Enzymol.* **133**, 27–42
4 Stanley, P.E. (1992) *J. of Bioluminescence and Chemiluminescence* **7**, 77–108.

5. Supplementary Information

5.1 Changes to previous version

- Editorial changes.

5.2 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Usage
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
🔍	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

5.3 Ordering Information

Product	Pack Size	Cat No.
ATP Bioluminescence Assay Kit CLS II	1600 assays (microwell plate); 800 assays (tube)	11 699 695 001
Luciferase (from *Photinus pyralis*)	1 mg	10 411 523 001
D(-)-Luciferin	50 mg	11 626 353 001
ATP, disodium salt, crystallized	5 g 10 g	10 127 523 001 10 127 531 001
ATP, disodium salt, crystals, special quality	1 g 5 g	10 519 979 001 10 519 987 001

Trademarks

Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

Disclaimer of License

For patent license limitations for individual products please refer to:
List of biochemical reagent products

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany