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

ATP Bioluminescence Assay Kit HS II

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Kit for the highly sensitive and quantitative detection of ATP by luciferase driven bioluminescence.

Cat. No. 11 699 709 001 1 kit
1,000 microplate assays, 500 tube assays

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	white	ATP Bioluminescence Assay Kit HS II, Luciferase reagent	Lyophilized	5 bottles, 10 ml each
2	red	ATP Bioluminescence Assay Kit HS II, ATP Standard	Lyophilized	5 bottles, 10 mg each
3	blue	ATP Bioluminescence Assay Kit HS II, Cell lysis reagent	▪ Ready-to-use solution bottled under sterile conditions. ▪ For the lysis of eukaryotic and prokaryotic cells.	1 bottle, 100 ml
4	green	ATP Bioluminescence Assay Kit HS II, Dilution buffer	▪ Ready-to-use solution free of ATP and microbial contaminations. ▪ For the dilution and reconstitution of Luciferase reagent and ATP Standard. ▪ For use as sample diluent.	1 bottle, 100 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	white	Luciferase reagent	Store at −15 to −25°C.
2	red	ATP Standard	
3	blue	Cell lysis reagent	Store at −15 to −25°C or +2 to +8°C. ⚠ ***Avoid contamination by microorgnisms or ATP by using autoclaved or heat-sterilized labware.***
4	green	Dilution buffer	

Storage Conditions (Working Solution)

ⓘ *See section,* **Reconstitution** *for additional information on preparing solutions.*

Reconstituted Solution	Storage and Stability
Luciferase reagent	▪ Store reconstituted reagent for 1 day at +15 to +25°C or 1 week at +2 to +8°C. ⓘ *Set up the standard curve each day, since a slight loss of light activity occurs during this time (approximately 20% after 5 days at +2 to +8°C).* ▪ For long-term storage, store at −15 to −25°C. ⓘ *Each freeze/thaw cycle reduces the luciferase activity, depending on the freezing conditions; shock freezing is the gentlest.* ⚠ ***Avoid repeated freezing and thawing.***
ATP Standard	▪ Store reconstituted ATP Standard 1 week (<5% degradation) at +2 to +8°C. ▪ Store at least 4 weeks (<5% degradation) at −15 to −25°C. ▪ Diluted ATP Standards are stable 8 hours on ice.

Reconstitution

Luciferase reagent

See section, **Storage Conditions (Working Solution)** *for additional information on storing solutions.*

1. Carefully add 10 ml of Dilution buffer (Bottle 4) to one bottle of Luciferase reagent (Bottle 1).

2. Incubate for 5 minutes at +2 to +8°C without stirring or shaking.

3. Rotate the bottle gently until the solution is homogeneous.
   ⚠ ***Do not shake.***

ATP Standard

See section, **Storage Conditions (Working Solution)** *for additional information on storing solutions.*
Each bottle of ATP Standard contains approximately 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.

1. Add the appropriate volume of Dilution buffer (Bottle 4) to dissolve the content of one Bottle of ATP Standard (Bottle 2) until a final concentration of 10 mg/ml or 16.5 mM, respectively, is achieved, for example, 960 µl to 9.60 mg ATP.

2. The ATP standard curve is prepared by serial dilutions of one ATP Standard with Dilution buffer.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment and reagents

- Autoclaved or heat-sterilized labware
- All commercially available luminometers (tube or microplate format)
- For microplate luminometers, use white or black microplates
- Automated injection systems provide the option to add reagents directly in the measurement position, and to start light signal integration after a constant, selectable delay.
  This opens the possibility to process even a large number of samples in a convenient and highly reproducible way.
- 100 mM Tris, 4 mM EDTA, pH 7.75

1.4. Application

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

- The kit uses the ATP dependency of the light emitting luciferase catalyzed oxidation of luciferin for the measurement of extremely low concentrations of ATP.
- Optimized for the highest sensitivity and provides the best results for the use in tube- and microplate-based luminometers.
- The preparation of an ATP calibration curve is facilitated using the supplied, stabilized ATP Standard stocks.
  The exact ATP content of the standard as indicated on the label is determined individually for each lot by HPLC and spectroscopy.
- Includes ready-to-use Cell lysis reagent 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, such as for metabolic studies or if coupled enzymatic assays are applied, use the ATP Bioluminescence Assay Kit CLS II which generates a constant light signal.*

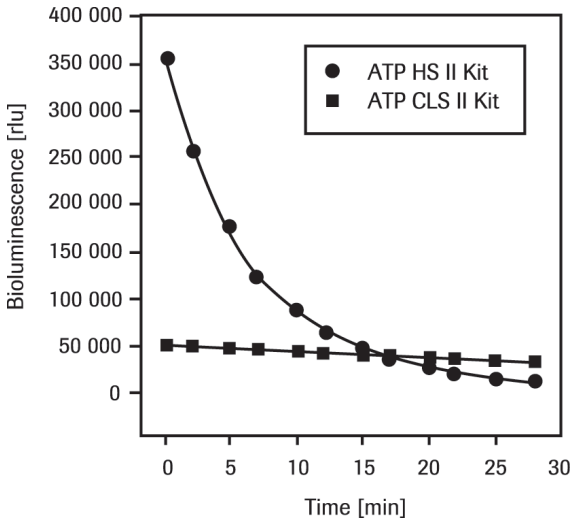

Fig. 1: Kinetics of light generation of the ATP Bioluminescence Assay Kit HS II and the ATP Bioluminescence Assay Kit CLS II. 10 pmol ATP in a volume of 50 µl was assayed with 50 µl Luciferase reagent in a black microplate.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Sample pH

The luciferase reaction's optimum pH is 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 Luciferase reagent is 25 mM.

General Considerations

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).

The ATP Bioluminescence Assay Kit HS II shows a rapid turnover of the sample ATP due to a high luciferase activity in the Luciferase reagent. This leads to an enhanced light output and to an improved sensitivity.

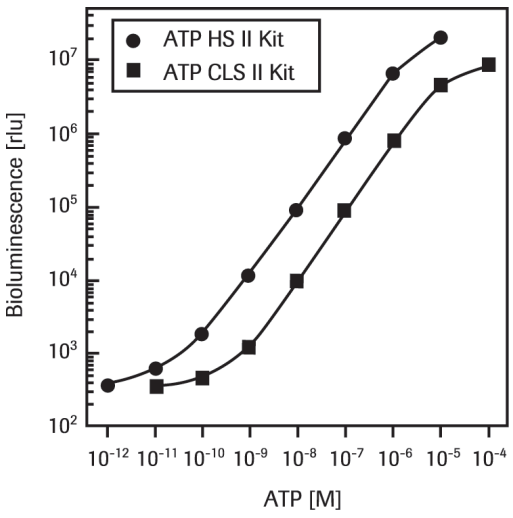

Fig. 2: Sensitivity range of the ATP Bioluminescence Assay Kit HS II and the ATP Bioluminescence Assay Kit CLS II. ATP dilutions in a volume of 50 µl were assayed with 50 µl Luciferase reagent in a black microplate on a Berthold LB 96 P luminometer. The light signal was integrated for 10 seconds after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

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 such as ethanol and butanol, and detergents or acids, such as TPA and PCA.
- Most of these extractants exhibit marked disadvantages such as 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 eukaryotic and prokaryotic cells with minimal influence on luciferase activity. However, some organisms such as yeast and algae are resistant at standard conditions. In these cases, the lysis reagent may also be used at elevated temperature (≤+100°C) or with prolonged incubation times.

The boiling method is one of the most efficient and universal ways to extract ATP from different types 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.

Lysis efficiency of the lysis reagent compared to boiling method

Boiling method = 100%.

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

2.2. Protocols

The standard protocols below are general guidelines only but can be used as a good starting point. 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	Procedure/Parameter	Microplate Format Volume [µl]	Tube Format Volume [µl]
1	If necessary, dilute samples with Dilution buffer (Bottle 4) to an appropriate ATP concentration. *The optimal detection range is between 10^{-7} to 10^{-10} M.* *The pH of the sample should be in the range of 7.6 to 8.0, see section,* **Sample Materials**.	50 (sample)	100 (sample)
2	Dilute ATP Standard with Dilution buffer (Bottle 4) by serial dilution in the range of 10^{-10} and 10^{-16} moles ATP (10^{-6} to 10^{-12} M).	50 (standard)	100 (standard)
3	Add Luciferase reagent to the samples/standards by automated injection. Start measurement after a 1 second delay and integrate for 1 to 10 seconds.	50 (reagent)	100 (reagent)
4	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

Step	Procedure/Parameter	Microplate Format Volume [µl]	Tube Format Volume [µl]
1	▪ Dilute cells to a concentration of 10^5 to 10^8 cells/ml. ▪ If the sample is too dilute, concentrate by centrifugation. For bacteria and yeast, use a maximum of 8,000 × *g* for 10 minutes; for somatic cells, use a maximum of 900 × *g* for 10 minutes. Discard the supernatant carefully. ▪ Resuspend pellet in a minimum volume of dilution buffer (50 to 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 (Bottle 3) directly from the filter, in a minimum volume. *For the dilution of the samples, 44 ml of Dilution buffer (Bottle 4) is available.*	25 (minimum)	50 (minimum)
2	Dilute ATP Standard with Dilution buffer (Bottle 4) by serial dilution in the range of 10^{-10} and 10^{-16} moles ATP (10^{-6} to 10^{-12} M).	25 (minimum)	50 (minimum)
3	▪ Add to the sample or ATP Standard the same volume of Cell lysis reagent (Bottle 3). ▪ Incubate 5 minutes 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 minutes at +100°C. ▪ Centrifuge sample at 10,000 × *g* for 60 seconds 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.*	–	–
4	Transfer the appropriate volume of sample/standard prepared in Step 3 into a microplate well or tube.	50	100
5	Add Luciferase reagent to the samples/standards by automated injection or by hand. Start measurement after a 1 second delay and integrate for 1 to 10 seconds.	50 (reagent)	100 (reagent)
6	Subtract the blank from the raw data and calculate ATP concentrations from a log-log plot of the standard curve data.	–	–

2.3. Parameters

Enzyme Kinetics

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 quickly from the start of the reaction, see section, **General Considerations, Figure 1**.
- In order to guarantee accuracy of the readings, the reagent volume, the injection conditions, and the onset of signal integration must be absolutely reproducible.
  These parameters must be considered, particularly when injections are done manually.

3. Troubleshooting

Observation	Possible cause	Recommendation
No signal or signal too weak.	Sample is too diluted.	Minimize volume prior to cell disruption, for example, 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 +2 to +8°C.
		Test for 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 for chelating agents. *The Luciferase reagent contains 10 mM Mg^{2}+.*
	Luciferase reagent destroyed.	Dissolve a new bottle of Luciferase reagent. *Do not stir or shake during dissolution. Once dissolved, store for a maximum of 5 days at +2 to +8°C.* ***Avoid repeated freezing/thawing.***
	pH of sample out of optimum range.	Adapt sample pH to 7.6 to 8.0.
	Measuring time too short.	Prolong the integration time with the luminometer.
	Use of wrong filter in the luminometer.	Do not use any filters during measurement.
Non-linear calibration curve.	Pipetting errors.	Pipette the serial dilutions carefully.
		Check precision of pipettes.
		Prepare the dilutions in triplicate.
	Crosstalk between individual wells of the microplate.	Use only white or preferably black microplates; do not use transparent microplates.
	Measuring time for the total microplate is too long; substrate is depleted in standards measured later in the assay.	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 the ATP Bioluminescence Assay Kit CLS II*.*
Too high background.	ATP contamination in water, buffers, or vials.	Use all Cell lysis reagent and Dilution buffer supplied in the kit. *Otherwise, check Cell lysis reagent and Dilution buffer for ATP contaminations.*
		Use only double-distilled 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, for example, 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, use a maximum of 8,000 × g; for somatic cells, use a maximum of 900 × g.*
		Reduce volume flow upon filtration of cells.
	No time-standardized cell harvesting conditions.	Standardize experimental time for all harvesting steps.
	Crosstalk between individual wells of the microplate.	Use only white or preferably black microplates; do not use transparent microplates.
	If an automated microplate luminometer is not used: Measuring time for the total microplate is too long; substrate or ATP is depleted in standards measured later in the assay.	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 the ATP Bioluminescence Assay Kit CLS II*.*

4. Additional Information on this Product

4.1. Test Principle

Living beings require a continual input of free energy for three major purposes:
- Performance of mechanical work in muscle contraction and other cellular movements.
- Active transport of molecules and ions.
- 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.

How this product works

1. Luciferase from *Photinus pyralis* (American firefly) catalyzes the following reaction:
   ATP + D-luciferin + O_2 → oxyluciferin + PP_i + AMP + CO_2 + light

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2. 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:
   light intensity = $(V_{max} \times C_{ATP}) / (K_m + C_{ATP})$

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

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4. This equation shows that the light output is directly proportional to the ATP concentration (C_{ATP}) and depends on the amount of luciferase (V_{max}) present in the assay.
   ⚠ ***Therefore, for maximum sensitivity, the sample ATP must be in a minimum volume and the Luciferase reagent must not be diluted.***

4.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
ATP Bioluminescence Assay Kit CLS II	1 kit, 1,600 microplate assays, 800 tube assays	11 699 695 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or 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.

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