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

# ATP Bioluminescence Assay Kit CLS II

**Version: 07**
Content Version: May 2021

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

**Cat. No. 11 699 695 001** 1 kit
1,600 microplate assays, 800 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 CLS II, Luciferase reagent | Lyophilized | 8 bottles, 10 ml each |
| 2 | red | ATP Bioluminescence Assay Kit CLS II, ATP Standard | Lyophilized | 4 bottles, Approximately 10 mg each |

## 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 | |

### 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.<br>*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).*<br>▪ For long-term storage, store at −15 to −25°C.<br>*Each freeze/thaw cycle reduces the luciferase activity, depending on the freezing conditions; shock freezing is the preferred method.*<br>***Avoid repeated freezing and thawing.*** |
| ATP Standard | ▪ Store reconstituted ATP Standard 1 week (<5% degradation) at +2 to +8°C.<br>▪ Store at least 4 weeks (<5% degradation) at −15 to −25°C.<br>▪ 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 double-distilled water 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 double-distilled water 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 double-distilled water.

## 1.3. Additional Equipment and Reagent required

### Standard laboratory equipment and reagents

- 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.*
- Double-distilled water
- 100 mM Tris, 4 mM EDTA, pH 7.75

## 1.4. Application

The ATP Bioluminescence Assay Kit CLS II is especially developed for applications where constant light signals are required:
- Kinetic studies of enzymes
- Metabolic studies
- Or if coupled enzymatic assays are applied.

If ATP determinations are started manually, the CLS II Kit provides high reproducibility due to the constant signal generation, see sections, **Sample Materials, Enzyme Kinetics, and Principle**.

*However, the sensitivity of the kit is by a factor of 10 lower as compared with the ATP Bioluminescence Assay Kit HS II* (Fig. 1), which is especially recommended for determinations in the high sensitivity range. The ATP Bioluminescence Assay Kit HS II contains also an efficient cell lysis reagent and can be used for the detection of ATP in microorganisms or animal cells.*

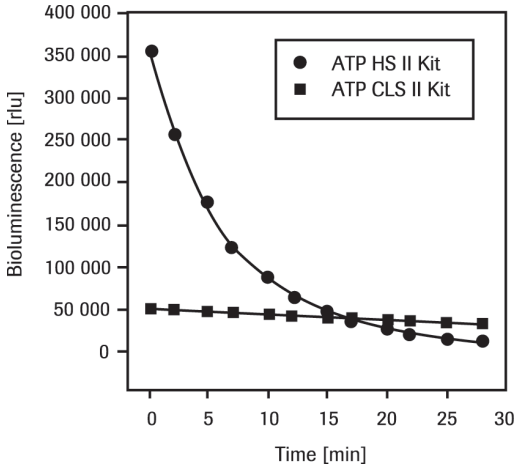


**Fig. 1:** Kinetics of light generation of the ATP Bioluminescence Assay Kit HS II and the ATP Bioluminescence Assay Kit CLS II. 10 pmol ATP in a volume of 50 µl was assayed with 50 µl Luciferase reagent in a black microplate on a Berthold LB 96 P luminometer.

# 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 40 mM.*

### General Considerations

#### Working range

The working range of the ATP Bioluminescence Assay Kit CLS II is between $10^{-6}$ and $10^{-11}$ 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 $10^{-11}$ M ($10^{-15}$ moles) (Fig. 2).

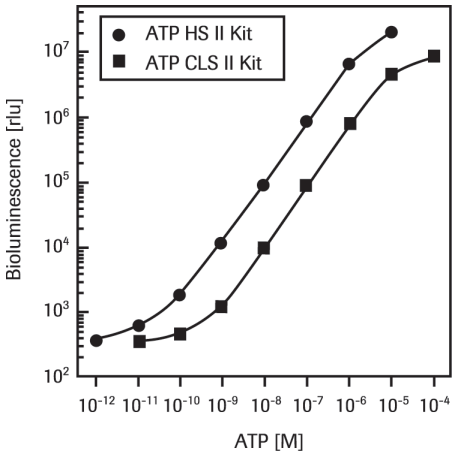


**Fig. 2:** Sensitivity range of the ATP Bioluminescence Assay Kit HS II and the ATP Bioluminescence Assay Kit CLS II. ATP dilutions in a volume of 50 µl were assayed with 50 µl Luciferase reagent in a black microplate on a Berthold LB 96 P luminometer. The light signal was integrated for 10 seconds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

## 2.2. Protocols

The standard protocols below are general guidelines only but can be used as a good starting point. A volume ratio of 50% reagent and 50% sample is optimal. When changing the assay volume, it is important that the concentration of the reagent in the assay remains the same.

### Determination of free ATP

| Step | Procedure/Parameter | Microplate Format Volume [µl] | Tube Format Volume [µl] |
|---|---|---|---|
| 1 | If necessary, dilute samples with double-distilled water or buffer to an appropriate ATP concentration.<br>*The optimal detection range is between $10^{-7}$ to $10^{-10}$ M.*<br>*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 double-distilled water by serial dilution in the range of $10^{-5}$ and $10^{-10}$ M. | 50 (sample) | 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 (no ATP) from the raw data and calculate ATP concentrations from a log-log plot of the standard curve data. | – | – |

## Determination of ATP from biological material

| Step | Procedure/Parameter | Required Volume for Microplate Format [µl] | Required Volume for Tube Format [µl] |
|---|---|---|---|
| 1 | ▪ Dilute cells to a concentration of $10^5$ to $10^8$ cells/ml.<br>▪ If the sample is too dilute, concentrate by centrifugation. For bacteria and yeast, use a maximum of 8,000 × *g*; for somatic cells, use a maximum of 900 × *g*. Discard the supernatant carefully.<br>▪ Resuspend pellet in a minimum volume of dilution buffer (50 to 100 µl).<br>▪ Concentration from large volumes can be performed by filtration through a 0.2 µm filter. Collect cells in a minimum volume. | 25 (minimum sample volume) | 50 (minimum sample volume) |
| 2 | Dilute ATP Standard with double-distilled water by serial dilution in the range of $10^{-5}$ and $10^{-10}$ M. | 25 (minimum sample volume) | 50 (minimum sample volume) |
| 3 | ▪ Add to the cell suspension, 9 volumes of boiling 100 mM Tris, 4 mM EDTA, pH 7.75.<br>▪ Incubate for another 2 minutes at +100°C.<br>▪ Centrifuge sample at 1,000 × *g* for 60 seconds and transfer the supernatant to a fresh tube.<br>▪ Keep samples on ice until measurement. | – | – |
| 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 and integrate for 1 to 10 seconds. | 50 (reagent) | 100 (reagent) |
| 6 | Subtract the blank (no ATP or no cells) 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 (constant light signal)

Due to the relatively low luciferase activity present in the assay mix, the ATP concentration can be considered to be constant with time.

- The reaction produces, for example, at an ATP concentration of $10^{-9}$ , a flow of photons of $5 \times 10^{7}$ photons $\times$ $ml^{-1} \times s^{-1}$.
- Assuming a quantum yield of 100%, this results in a reduction of ATP concentration of 0.5% per minute, that is, under optimum experimental conditions, a nearly constant light signal at a defined ATP concentration is obtained (Fig. 1).

*Therefore, when performing the assay manually, there is sufficient time for addition of the reagent, placing the sample into the luminometer, and starting the measurement.*

# 3. Troubleshooting

| Observation | Possible cause | Recommendation |
|---|---|---|
| No signal or signal too weak. | ATP content in the sample is very low. | Use ATP Bioluminescence Assay Kit HS II* for detection of ATP with highest sensitivity. |
| | 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.<br>*The Luciferase reagent contains 20 mM $Mg^{2}$+.* |
| | Luciferase reagent destroyed. | Dissolve a new bottle of Luciferase reagent.<br>*Do not stir or shake during dissolution. Once dissolved, store for a maximum of 5 days at +2 to +8°C.*<br>***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 constant for all samples (maximum 5 minutes). |
| Too high background. | ATP contamination in water, buffers, or bottles. | Use only double-distilled water for the preparation of reagents and 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.<br>*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. |

# 4. Additional Information on this Product

## 4.1. Test Principle

The determination of ATP using bioluminescence is a well established technique.

- Uses the ATP dependancy of the light emitting luciferase catalyzed oxidation of luciferin for the measurement of extremely low concentrations of ATP.
- The ATP Bioluminescence Assay Kit CLS II kit is especially optimized for easy use in tube luminometers as well as in microplate-format luminometers.
- The kit exhibits a constant light signal sustaining for several minutes.
- Especially suited for kinetic studies and ATP determinations in coupled enzymatic reactions.
- The preparation of an ATP calibration curve is facilitated using the stabilized ATP Standard stock provided. The exact ATP content of the standard as indicated on the label is determined individually for each lot by HPLC and spectroscopy.

### How this product works

- Luciferase from *Photinus pyralis* (American firefly) catalyzes the following reaction:
  ATP + D-luciferin + $O_2$ → 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:
  light intensity = $(V_{max} \times C_{ATP}) / (K_m + C_{ATP})$
- At low ATP concentrations ($C_{ATP} << K_m$), the formula simplifies to:
  light intensity = $V_{max} \times C_{ATP}/K_m$
- 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.

# 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 HS II | 1 kit, 1,000 microplate assays, 500 tube assays | 11 699 709 001 |

## 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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