ATP Kit SL

Monitoring of ATP in e.g. assays of enzymes and metabolites, cell lysis or platelet aggregation

- Fast assay: Results within minutes
- Stable light: Half-life >1 h
- Detection limit: $10^{-15}$ mol or $10^{-12}$ mol/L ATP
- Reliable: ATP Standard in liquid form
- Flexible: Choice of µ-plate & cuvette methods

Leader in luminescent ATP-assays
ATP Kit SL

Intended use
ATP Kit SL is intended for monitoring of adenosine triphosphate (ATP) in the range $10^{-12}$ - $10^{-6}$ mol/L. The low decay rate of the light emission (around 0.9 % per min) is due to a low consumption of ATP in the firefly luciferase reaction, and a luciferase activity remaining unchanged during the measurement. This allows enzymatic formation or degradation of ATP and cellular release of ATP to be monitored by measuring the light intensity

Applications
1. Assays of enzymes and metabolites participating in ATP converting reactions.
2. Monitoring of oxidative phosphorylation and photophosphorylation.
3. ATP release during cell lysis.
4. ATP release during platelet aggregation.

Assay principles
Firefly luciferase catalyses the following reaction:

$$\text{luciferase} \quad \text{ATP} + \text{D-luciferin} + \text{O}_2 \rightarrow \text{AMP+ PPI + oxyluciferin + CO}_2 + \text{light}$$

The assay has been optimised to give a stable light at all ATP levels up to $10^{-6}$ mol/L.

Instruments
The stable light makes it possible to use manual single tube luminometers, automatic tube luminometers or microplate luminometers. The detection limit depends on luminometer sensitivity.

Kit contents
1. The kit is intended for 200-1000 assays depending on the final assay volume (0.2-1 mL).
2. ATP Reagent SL. 4 vials of lyophilised reagent containing D-luciferin and luciferase.
3. Diluent C 10 mL. 4 vials for reconstitution of ATP Reagent SL.
4. ATP Standard 5 mL. 4 vials containing $10^{-5}$ moles/L of ATP.
5. Tris-EDTA Buffer 2x100 mL. 2 bottles containing 0.1 mol/L Tris(hydroxymethyl) aminomethane, 2 mmol/L EDTA and adjusted to pH 7.75 with acetic acid.

Assay procedure using internal ATP Standard
The light emission is measured before and after the addition of a known amount of ATP. This strongly increases the reliability of the assay and makes it possible to express ATP results in moles rather than rlu or other non-chemical units. The procedure below is for tube luminometers. The assay can also be automatically performed in microplate luminometers.

1. Add sample and Tris-EDTA Buffer to give a total volume of 0.8 mL in the cuvette.
2. Add 0.2 mL ATP Reagent SL.
3. Monitor the light emission corresponding to sample ATP, $I_{smp}$.
5. Measure the light emission corresponding to sample plus standard ATP, $I_{smp+std}$.

Calculations:
Calculate the sample ATP concentration in the cuvette by the following equation:

$$\text{ATP}_{smp} = 10^{-7} \times \frac{I_{smp}}{I_{smp+std} - I_{smp}}$$

The factor $10^{-7}$ is the concentration of ATP Standard in the cuvette.


Product characteristics
Detection limit: $10^{-12}$ mol/L or $10^{-15}$ mol ATP
No. of determinations (cuvettes): 200
No. of determinations (microplate): 1000

Ordering info
Article No: 144-041

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ATP Kit SL
Article No. 144-041

Intended use
ATP Kit SL is intended for monitoring of ATP (adenosine triphosphate) over the range of 10^{-12} - 10^{-6} mol/L. The low decay rate of the light emission (around 0.9%/min) is due to a low consumption of ATP in the firefly luciferase reaction and a luciferase activity that is unchanged during the time of the measurement. Under these circumstances measurement of the intensity of the light emission allows continuous monitoring of formation or degradation of ATP in enzymatic reactions or release of ATP from cells. Thus assays can be performed in much the same way as NAD(P)H is spectrophotometrically or fluorometrically monitored in assays based on dehydrogenase reactions. Furthermore the stable light makes assays even of a constant ATP level more convenient and reliable, particularly when using manual luminometers. If a higher sensitivity is required BioThema has two other reagents allowing the detection of 10^{-17} mol and 10^{-18} mol, resp. Please cf. our website: www.biothema.com

Assay principles
Firefly luciferase catalyses the following reaction:

\[
\text{luciferase} \quad \text{ATP} + \text{D-luciferin} + \text{O}_2 \rightarrow \text{AMP} + \text{PPi} + \text{oxyluciferin} + \text{CO}_2 + \text{light}
\]

The assay has been optimised to give a stable light at all ATP levels up to 10^{-6} mol/L. The reagent contains D-luciferin, magnesium ions, PPi (inorganic pyrophosphate) and BSA (bovine serum albumin).

Kit contents
1. ATP Reagent SL. 4 vials of lyophilised reagent containing D-luciferin, luciferase and stabilizers. (Box A)
2. Diluent C 10 mL. 4 vials containing diluent to reconstitute ATP Reagent SL. (Box A)
3. ATP Standard 4 mL. 5 vials containing 10^{-5} moles/L of ATP. (Box A)
4. Tris-EDTA Buffer 100 mL. 2 bottles containing 0.1 mol/L Tris(hydroxymethyl) aminomethane, 2 mmol/L EDTA and adjusted to pH 7.75 with acetic acid. (Box B)

The kit boxes can be divided upon arrival and stored separately. Box A containing ATP Reagent SL should be stored at -18 °C or lower, and Box B containing Tris-EDTA Buffer can be stored at +4 °C. However, during normal transport times the kit is not affected by ambient temperature. If used within a week the kit may be stored at +4 °C. The kit and the individual components are labelled with expiry date assuming storage at indicated temperature.

Reagent reconstitution
The ATP Reagent SL is reconstituted by adding the entire content of the Diluent C vial (10 mL) to the reagent vial. It is very important to avoid contamination. Please use a clean pair of tweezers to remove the rubber stopper from the ATP Reagent vial. Pour the diluent gently into the reagent vial to dissolve the lyophilised reagent. Subsequently pour the solution back into the Diluent C vial. Once reconstituted the reagent must be protected from light. In reconstituted ATP Reagent the luciferase activity slowly decays, e.g. storage at +4 °C decreases the activity of the ATP Reagent by 0.7% per day. However, by calibrating the assays with ATP Standard makes it possible to use the ATP reagent for several days at +4 °C or for 1 month at -18 °C. Reagents should be allowed to attain room temperature before the assay (the optimum temperature for the luciferase reaction is around 25 °C).

Instrumentation
The stable light makes it possible to use manual single tube luminometers, automatic tube luminometers or microplate luminometers. The detection limit obviously depends on the luminometer. With most luminometers 10^{-15} moles of ATP can be detected.

Assay procedure using internal ATP Standard
In this procedure a known amount of ATP is added in the assay of each individual sample. This strongly increases the reliability of the assay and makes it possible to express ATP results in moles rather than rlu or other non-chemical units. The assay can also be performed using an external ATP. However, this is a less reliable procedure in most cases. Please consult BioThema for advice, if you are considering using external ATP Standard. The use of ATP Standard as described below
compensates for variation of reaction conditions affecting the luciferase activity and for instrument and microplate variations. The fact that different media give different light emission with the same amount of ATP is therefore not a problem.

A Tube luminometer
1. Add sample and Tris-EDTA Buffer to give a total volume of 0.8 mL in the cuvette.
2. Add 0.2 mL ATP Reagent SL
3. Measure the light emission corresponding to sample ATP, I_{amp}.
5. Measure the light emission corresponding to sample plus standard ATP, I_{amp+std}.
6. Calculate the sample ATP concentration in the cuvette by the following equation:

   \[ \text{ATP}_{\text{amp}} = 10^{-7} \times \frac{I_{\text{amp}}}{I_{\text{amp+std}} - I_{\text{amp}}} \]

   The factor \(10^{-7}\) is the concentration (mol/L) of ATP Standard in the cuvette (10 µL \(10^{-5}\) mol/L in a total reaction volume of 1 mL).

B Microplate luminometer
1. Add sample and Tris-EDTA Buffer to give a total volume of 160 µL in each well.
2. Add 40 µL ATP Reagent SL
3. Measure the light emission corresponding to sample ATP, I_{amp}.
4. Add 10 µL of ATP Standard diluted 1/5 in Tris-EDTA Buffer.
5. Measure the light emission corresponding to sample plus standard ATP, I_{amp+std}.
6. Calculate the sample ATP concentration in the well by the following equation:

   \[ \text{ATP}_{\text{amp}} = 10^{-7} \times \frac{I_{\text{amp}}}{I_{\text{amp+std}} - I_{\text{amp}}} \]

   The factor \(10^{-7}\) is the concentration (mol/L) of ATP Standard in the well (10 µL \(0.2 \times 10^{-5}\) mol/L in a total reaction volume of 0.2 mL).

Procedural notes
1. The assay is linear from the detection limit, which with most luminometers is around \(10^{-12}\) mol/L, and up to \(10^{-6}\) mol/L. In the final assay mixture the sample ATP concentration should be <10% of the ATP Standard. Otherwise the dilution effect from the addition of the ATP Standard will result in a too low value of \(I_{\text{amp+std}} - I_{\text{amp}}\) resulting in an overestimation of sample ATP. Maximum accuracy is generally obtained if the sample ATP concentration in the final reaction mixture is \(10^{-10}\) to \(10^{-8}\) mol/L. The sample volume should also be chosen to avoid unnecessary inhibition of light emission from sample components (e.g. extractant). An inhibition of up to 50% is normally not a problem, since the effect is compensated for by the internal ATP Standard.

2. The ATP Standard volume should be as low as possible with a retained precision, since the dilution effect coming from the addition of ATP Standard otherwise can’t be neglected. With a total reaction volume of 1 mL it is acceptable to use 10 µL. With a total reaction volume of 200 µL an ATP Standard volume of 10 µL is actually somewhat too high, but has to be accepted since lower volumes of ATP Standard can’t normally be added with high precision. The dilution effect can be estimated by adding 10 µL Tris-EDTA Buffer to the assay mixture measuring the decrease of the light emission. The effect on \(I_{\text{amp+std}}\) can then be mathematically compensated for.

3. The light emission should be measured as soon as possible after addition of reagents (ATP Reagent and ATP Standard, resp.). Maximum light emission is normally obtained within a few seconds and will then start to decay by 0.9%/min.

4. Every series of assays should include at least two blanks in the beginning and at the end. Blanks should contain all reagents added in the preparation of samples (e.g. extractants used for releasing ATP from cells) and should be assayed and calculated exactly as normal samples. Blanks calculated by the formula above are subtracted from sample ATP values calculated in the same way. The ATP concentration in original biological sample is subsequently calculated by multiplying with the dilution in the assay and in the preparation of the sample for the assay.