# ATP Assay Kit (Chemiluminescent)

ATP Assay Kit (Chemiluminescent) is a detection kit for the quantification of ATP in tissue and cell lysates samples.

Catalog number: ARG82120

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## INTRODUCTION

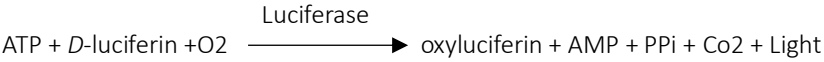
Adenosine triphosphate (ATP) is an organic compound that provides energy to drive many processes in living cells, e.g. muscle contraction, nerve impulse propagation, and chemical synthesis. Found in all known forms of life, ATP is often referred to as the "molecular unit of currency" of intracellular energy transfer. When consumed in metabolic processes, it converts either to adenosine diphosphate (ADP) or to adenosine monophosphate (AMP). Other processes regenerate ATP so that the human body recycles its own body weight equivalent in ATP each day. It is also a precursor to DNA and RNA, and is used as a coenzyme.

From the perspective of biochemistry, ATP is classified as a nucleoside triphosphate, which indicates that it consists of three components: a nitrogenous base (adenine), the sugar ribose, and the triphosphate.

Biochemical functions including: Intracellular signal transduction, DNA and RNA synthesis, Amino acid activation in protein synthesis, ATP binding cassette transporter, Extracellular signaling and neurotransmission and Protein solubility. [Wikipedia: Adenosine triphosphate]

## PRINCIPLE OF THE ASSAY

This ATP Assay Kit (Chemiluminescent) Assay Kit provides a rapid and easy method to measure intracellular ATP in tissue and cell lysates samples. The assay working reagent lyses cells to release ATP, The luciferase in the assay working reagent immediately reacts with the Substrate D-luciferin to produce light. This reaction is then measured in a plate luminometer. The concentration of ATP in the sample is then determined by comparing the RLU of samples to the standard curve.




## MATERIALS PROVIDED & STORAGE INFORMATION

| Component | Quantity | Storage information |
|---|---|---|
| Assay Buffer | 10 ml (Ready to use) | -20°C |
| Substrate (*D*-luciferin) | 120 µl | -20°C |
| ATP Enzyme | 120 µl | -20°C |
| Standard (3 mM ATP) | 100 µl | -20°C |

The kit is shipped on ice. Store all components at -20°C in dark. Shelf life of six months after receipt.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Plate Luminometer.
- White Opaque 96-well Microplate.
- Pipettes and pipette tips
- Deionized or distilled water.

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The kit is shipped on ice. Store all components at -20°C in dark. Shelf life of six months after receipt.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Before each assay, it is important to check that enzyme preparations and assay buffers do not contain free phosphate.
- Assay Buffer and Substrate should be equilibrated to room temperature (RT) before use. Thaw ATP enzyme on ice or at 4°C before use.
- Store unused reagents including the enzyme at -20°C.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Assays can be carried out in a tube or in a microplate.
- It is recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue sample:

1. Weigh the tissue and add 200 µL of cold PBS per 20 mg of tissue.
2. Homogenize the sample on ice using a Polytron-type homogenizer.
3. Spin at 12,000 g for 5 min at 4°C.
4. Collect the supernatant and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: 10 µl of sample is requested for the further assay. It is suggested to make a series dilution of tissue sample in PBS before assay to let the RLU within the standard curve range. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the assay buffer before assay.

Cultured cell sample:

1. For suspension cells, transfer 10 µl of the cultured cells (with around $10^3$-$10^4$ cells) directly into a white opaque 96 well plate.
2. For adherent cells, culture $10^3$-$10^4$ cell directly in a sterile and tissue culture available white opaque 96-well tissue culture plate. Or resuspend around $10^3$-$10^4$ cells in 10 µl of culture medium and transfer into a white opaque 96-well plate.

## REAGENT PREPARATION

- **Standard:** Adding 5 µL of 3 mM ATP standard stock into 495 µL of distilled water to yield 500 µL of 30 µM ATP standard solution, mix well. (Note: for cultured cell samples dilute ATP in culture media) Dilute the 30 µM ATP standard solution with distilled water (or culture media for cultured cell samples) to yield standard concentration as 30 µM, 24 µM, 18 µM, 12 µM, 9 µM, 6 µM and 3 µM. The distilled water (or culture media for cultured cell samples serves as zero standard (0 µM),

| Standard No. | Standard Conc. µM | Distilled water (or medium) (µl) | Standard (µl) |
|---|---|---|---|
| S1 | 30 | 495 | 5 µl of 3 mM Stock |
| S2 | 24 | 10 | 40 µl of S1 |
| S3 | 18 | 20 | 30 µl of S1 |
| S4 | 12 | 30 | 20 µl of S1 |
| S5 | 9 | 35 | 15 µl of S1 |
| S6 | 6 | 40 | 10 µl of S1 |
| S7 | 3 | 45 | 5 µl of S1 |
| S0 | 0 | 50 | 0 |

- **Assay working reagent-** Fresh preparation is recommended. For each 96-well, mix 95 µl of Assay Buffer with 1 µl of Substrate and 1 µl of ATP Enzyme. 90 µl of this Assay working reagent is requested for each well. Avoid exposure to light.

  **Note:**

  Assay Buffer and Substrate should be equilibrated to room temperature (RT) before use. Thaw ATP enzyme on ice or at 4°C before use.

## ASSAY PROCEDURE

Assay Buffer and Substrate should be equilibrated to room temperature (RT) before use. Thaw ATP enzyme on ice or at 4°C before use. Each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use.

1. Add 10 µl of Standard (S0-S7) in duplicate into the wells in a white opaque 96-well microplate.
2. Add 10 µl of samples into in the white opaque 96-well microplate.
   (For the cell cultured directly in a white opaque 96-well tissue culture plate, remove the culture medium before adding the Assay working reagent.)
3. Add 90 µl of the Assay working reagent to each well. Avoid exposure to light.
4. Gently tap the plate to ensure thorough mixing. Avoid exposure to light.
5. Read the luminescence of each microwell on a plate luminometer immediately. It is recommended reading the luminescence within 1 min after adding the Assay working reagent.

Summary:

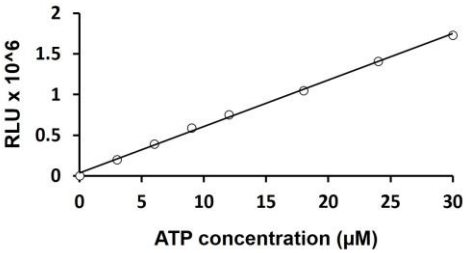
| | Samples | Cell cultured in the well (Remove medium before assay) | Standard |
|---|---|---|---|
| Sample | 10 µl | - | - |
| Standard (S0-S7) | - | - | 10 µl |
| Add 90 µl of the Assay working reagent to each well. | | | |
| Read the luminescence on a plate luminometer immediately. | | | |

Note: Since the signal of the reaction decreases by ~1% each minute, for most accurate results, care should be taken that the time between adding the Assay working reagent and luminescence reading is the same for all samples and standards.

## CALCULATION OF RESULTS

1. Calculate the average RLU (relative light units) values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean RLU obtained from each standard against its concentration with RLU value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean RLU value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the RLU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve or linear curve fit.
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

## EXAMPLE OF ASSAY



## QUALITY ASSURANCE

### Sensitivity

The minimum detectable dose (MDD) of ATP was:

0.1 µM