Technical Bulletin

# ATP Assay Kit

**Catalogue Number MAK473**

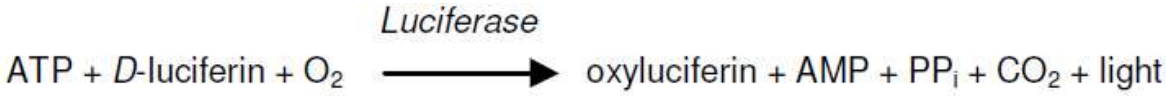
## Product Description

Adenosine 5'-triphosphate (ATP) is the chemical energy source for cellular metabolism and is often referred to as "energy currency" of the cell. ATP is produced in living cells during photosynthesis and cellular respiration and consumed in cellular processes including biosynthetic reactions, motility, and cell division. ATP is a key indicator of cellular activity and has been utilized as a measure of cell viability and cytotoxicity in research and drug discovery.

The ATP Assay Kit provides a rapid method to measure intracellular ATP. The single working reagent lyses cells to release ATP, which in the presence of luciferase, immediately reacts with the substrate D-luciferin to produce light. The light intensity is a direct measure of intracellular ATP.

This non-radioactive, homogeneous cell-based assay is performed in microplates. The reagent is compatible with all liquid handling systems for high-throughput screening applications in 96-well and 384-well plates.

The kit can detect as low as 0.02 µM ATP, or a single cell can be quantified. The kit is suitable for ATP determination in cells, tissue, and other biological samples.




## Components

The kit is sufficient for 100 bioluminescent assays in 96-well plates.

- Assay Buffer — 10 mL
  Catalogue Number MAK473A
- Substrate — 120 µL
  Catalogue Number MAK473B
- ATP Enzyme — 120 µL
  Catalogue Number MAK473C
- Standard (3 mM ATP) — 100 µL
  Catalogue Number MAK473D

## Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Luminometer
- White opaque flat-bottom 96-well or 384-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes

For tissue samples:

- Dounce tissue grinder set
  (Catalogue Number D9063 or equivalent)
- Phosphate Buffered Saline (PBS) (Catalogue Number P3813 or equivalent)
- Microcentrifuge capable of RCF ≥ 12,000 × *g*

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

## Preparation Instructions

Briefly centrifuge small vials prior to opening.

Bring Assay Buffer and Substrate to room temperature. Thaw ATP Enzyme on ice or at 4 °C. Store unused reagents including the enzyme at -20 °C.

## Procedure

All samples and standards should be run in duplicate.

### Sample Preparation

#### Tissue Samples

1. Homogenize 20 mg of sample in 200 µL of cold phosphate buffered saline (PBS).
2. Centrifuge at 12,000 × *g* for 5 minutes to pellet any debris.
3. Transfer 1-10 µL supernatant to each well and bring the total volume to 10 µL with PBS.
4. Test several doses of the Sample and choose the readings that are within the standard curve range for ATP calculation.

#### Suspension Cells

Transfer 10 µL of the cultured cells ($10^3$ - $10^4$) into a white opaque 96-well plate.

#### Adherent Cells

1. Culture $10^3$ - $10^4$ cells in a white opaque microplate.
2. At the time of assay, remove the culture medium **immediately** before adding Reaction Mixture (see Step 1 of Assay Reaction section).

### Standard Curve Preparation

1. Prepare a 30 µM ATP Standard by mixing 5 µL of 3 mM ATP Standard and 495 µL of purified water (for cell culture samples dilute ATP in culture media rather than purified water).
2. Prepare ATP standards in 1.5 mL microcentrifuge tubes according to Table 1.

**Table 1.**
Preparation of ATP Standards

| Well | 30 µM ATP Standard | Purified Water | ATP (µM) |
|---|---|---|---|
| 1 | 50 µL | - | 30 |
| 2 | 40 µL | 10 µL | 24 |
| 3 | 30 µL | 20 µL | 18 |
| 4 | 20 µL | 30 µL | 12 |
| 5 | 15 µL | 35 µL | 9 |
| 6 | 10 µL | 40 µL | 6 |
| 7 | 5 µL | 45 µL | 3 |
| 8 | - | 50 µL | 0 |

3. Mix well and transfer 10 µL of each Standard into separate wells of a white opaque 96-well plate.

### Reaction Mixture

For each well, prepare 97 µL of Reaction Mixture according to Table 2.

**Table 2.**
Preparation of Reaction Mixture

| Reagent | Volume |
|---|---|
| Assay Buffer | 95 µL |
| Substrate | 1 µL |
| ATP Enzyme | 1 µL |

## Assay Reaction

Note: The signal of the reaction decreases by ~1% each minute. For most accurate results, care should be taken that the time between adding the Reaction Mixture and luminescence reading is the same for all samples and standards. Use of a multi-channel pipettor is recommended.

1. Add 90 µL of Reaction Mixture to each well.
2. Mix by tapping the plate.
3. **Immediately** (within 1 minute after adding Reaction Mixture) read luminescence (RLU) on a luminometer.

# Results

1. Subtract the blank (Standard #8) luminescence (RLU) value from the remaining standard values.
2. Plot the adjusted values against standard concentrations and determine the slope.
3. Calculate the ATP concentration of Sample using the below equation:

ATP (µM) =

$$\frac{R_{SAMPLE} - R_{BLANK}}{Slope\ (\mu M^{-1})}\ x\ DF$$

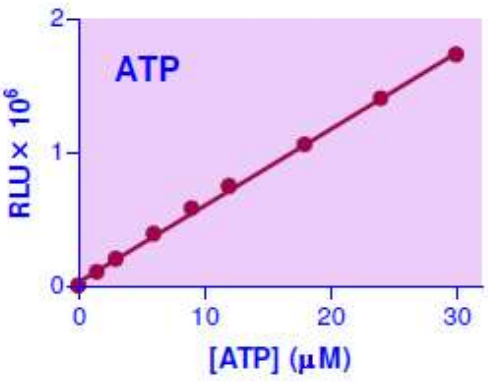
where:

$R_{Sample}$ = Luminescence (RLU) value of Sample

$R_{Blank}$ = Luminescence (RLU) value of Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Typical ATP Standard curve in purified water

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