Technical Bulletin

ATP Assay Kit

Catalogue Number MAK603

Product Description

Adenosine triphosphate (ATP) is a crucial nucleotide triphosphate that serves as the primary energy carrier in all living organisms. It is produced in the mitochondria and plays a vital role in driving numerous cellular processes, including metabolism, muscle contraction, and signal transduction[1]. Disruptions in ATP production can lead to various metabolic disorders and energy-related diseases, highlighting the importance of accurately measuring ATP levels in biological samples[2,3].

The ATP Colorimetric/Fluorometric Assay Kit offers a simple and friendly method for quantifying ATP concentrations. This assay is based on a series of enzymatic reactions resulting in the formation of a compound that exhibits both colorimetric (570 nm) and fluorometric (λ_{ex}=535 / λ_{em}=587nm) properties. The intensity of the signal is directly proportional to the ATP concentration in the sample.

This kit is suitable for the quantitative determination of ATP in various biological samples, including tissues and cell cultures, and is valuable for evaluating the effects of drugs on cellular energy metabolism.

A convenient assay calculator is available on the MAK603 webpage.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer — 25 mL
  Catalogue number MAK603A
- ATP Probe, in DMSO — 0.25 mL
  Catalogue number MAK603B
- ATP Converter — 5 µl
  Catalogue number MAK603C
- Developer Mix — 0.25 mL
  Catalogue number MAK603D
- ATP Standard, 1.0 µmole — 1.2 mg
  Catalogue number MAK603E

Reagents and Equipment Required but Not Provided

- 96-well flat-bottom plate
  - Clear plates for colorimetric assays (Catalogue number M2936 or equivalent)
  - Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent)
  - Cell culture or tissue culture treated plates are not recommended.
- Fluorescence multi-well plate reader that can read 96-well plates at wavelength of λ_{ex}=535 nm/λ_{em}=587.
- Pipettors and Pipettes
- Vortex Mixer
- NP-40 (Catalogue number 492018 or equivalent)
- Liquid N_2 (optional for long-term sample storage)
- Amicon 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter (optional for protein-containing samples)

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 product is shipped on dry ice. Store at -20°C upon receipt.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Assay Buffer (MAK603A)

Allow buffer to come to room temperature before use.

ATP Probe (MAK603B)

Allow the ATP Probe to come to room temperature before use. Use within 6 months. For the fluorescence assay, it is preferable to dilute an aliquot of the ATP Probe 5 to 10-fold with Assay Buffer prior to use. This will reduce the background of the fluorescence assay.

ATP Converter (MAK603C)

Thaw the solution at room temperature for 5 minutes and dilute with 350 µL of cold Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Developer Mix (MAK603D)

Store at –20 °C. Keep on ice while in use.

ATP Standard (MAK603E)

Reconstitute the 1.2 mg ATP with 200 µL of cold ultrapure water to generate a 10 mM ATP Standard Solution. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice during use.

Procedure

All samples and standards should be run in at least duplicates. A convenient customer calculator can be found on the product webpage.

ATP Standards for Colorimetric Detection

1. Dilute 10 µL of the 10 mM (10 nmol/µl) ATP Standard Solution with 90 µL of ultrapure water to prepare a 1 mM (1 nmol/µl) Standard Solution.
2. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM (1 nmol/µl) standard solution into a 96-well plate, generating 0 (assay blank), 2, 4, 6, 8, and 10 nmol/well Standards.
3. Add Assay Buffer to each well to bring the final volume to 50 µL.

ATP Standards for Fluorometric Detection

1. Dilute 10 µL of the 1 mM (1 nmol/µl) ATP Standard Solution with 90 µL of the ultrapure water to prepare 0.1 mM (100 pmol/µl) ATP Standard Solution or with 990 µL of ultrapure water to prepare a 0.01 mM (10 pmol/µl) Standard Solution.
2. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM standard solution into a 96 well plate, generating 0 (blank), 200, 400, 600, 800, and 1000 pmol/well standards or of the 0.01 mM standard solution into a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 pmol/well standards.
3. Add Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation

Sample preparation should be performed promptly and on ice to preserve endogenous ATP and minimize ATPase activity. Always use fresh samples when possible. For long-term storage, snap-freeze samples in liquid nitrogen and store at ≤−80°C, avoiding repeated freeze-thaw cycles.

Cultured Cells

Prepare a lysis buffer by adding NP-40 (Catalogue number 492018) to the Assay Buffer at a final concentration of 0.5%. Maintain a consistent sample-to-buffer ratio of approximately 1 million cells per 100 µL of

lysis buffer. Gently mix the solution and immediately transfer it to a cold centrifuge set to 4°C. Centrifuge for 5 minutes at 13,000 g. Transfer the supernatant (SUP) to a 10 kDa cutoff Amicon and centrifuge again for another 5 minutes at 13,000 g to effectively remove ATP-consuming enzymes. Avoid prolonged centrifugation, as ATP is unstable and will hydrolyze over time. The samples are now ready for ATP measurement.

Note: Use the lysis buffer for all subsequent steps, including standard preparation, sample preparation, and reaction mix formulation.

Tissue Samples

Use fresh tissue and keep it cold during preparation. Perform homogenization on ice to ensure effective lysis. Do not add NP-40 to the Assay Buffer. Instead, add the Assay Buffer at a ratio of 100 µL per 10 mg of tissue. Achieve lysis through homogenization. The samples are then ready for ATP measurement.

Important Considerations

- Work on ice, probe excluded.
- Include a sample blank processed identically to the experimental samples but omit the ATP-to-signal conversion step (e.g., ATP Converter). The background reading from the sample blank should be subtracted from all sample readings to account for background interference in the calculation process.
- For unknown samples, it is advisable to conduct a preliminary experiment using multiple dilutions. This initial test will help determine the appropriate dilution to use and whether to employ the fluorescent or colorimetric method for ATP measurement. There is no need for replicates or spike samples during this preliminary assessment.
- Make sure OD/FLU fall within the range of the standard curve. Do multiple dilution.

Add 2–50 µL of samples to duplicate wells of a 96-well plate. Adjust the final volume of each well to 50 µL using Assay Buffer.
A sample blank control should be performed for each reaction by omitting the ATP Converter. The background should be subtracted from all readings.

Assay Reaction

1. Set up the Master Reaction Mix according to Table 1 or Table 2. 50 µL of the Master Reaction Mix is required for each reaction (well). It is recommended to prepare an additional reaction mix for every six reactions. Therefore, for an experiment involving six samples, you should prepare enough mix for seven samples.

Table 1.
Master Reaction Mix for Colorimetric Assay

Reagent	Samples and Standards	Sample Blank
ATP Assay Buffer	44 µL	46 µL
ATP Probe	2 µL	2 µL
ATP Converter	2 µL	–
Developer Mix	2 µL	2 µL

Note: The fluorometric assay is 10–100 times more sensitive than the colorimetric assay.

Table 2.
Master Reaction Mix for Fluorometric Assay

Reagent	Samples and Standards	Sample Blank
ATP Assay Buffer	45.8 µL	47.8 µL
ATP Probe	0.2 µL	0.2 µL
ATP Converter	2 µL	–
Developer Mix	2 µL	2 µL

2. Add 50 µL of the Master Reaction Mix to each sample, ATP standard, and background control wells and mix.
3. Incubate the plate for 30 minutes with shaking (optional) at room temperature. Protect the plate from light during the incubation.
4. For colorimetric assay, measure the absorbance at 570 nm (A570). For fluorometric assay, measure fluorescence intensity (λ_{ex}=535/λ_{em}=587 nm).

Results

Calculations

Correct Assay Values: The background for the assay is the value obtained for the 0 (blank assay) ATP Standard. Blank assay values can be significant and must be subtracted from all readings (sample, sample spike, sample blank, and sample standards).

Correct Samples Values: Subtract the sample background control value from the sample readings (including sample spiked if relevant).

Use the values obtained from the appropriate ATP standards to plot a standard curve. The amount of ATP present in the samples may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Table 3.
Definitions of Sample Types

Name	Description
Blank assay	Assay background
Sample blank	Sample background
Sample spike	Sample with added ATP at known level
Sample	Sample test

Concentration of ATP

C = DF * S_a/S_v

DF = Dilution factor for the sample, applied if the sample is diluted to ensure it falls within the range of the standard curve before setting up the reaction wells.

S_a = Quantity of ATP in the unknown sample calculated from the standard curve (nmole or pmole).

S_v = Sample volume added into the wells (µL).

C = Concentration of ATP in sample (nmole/µL or pmole/µL)

ATP molecular weight is 507.18 g/mole.

For spiked samples, correct for interference by using the following equation (for colorimetric and fluorometric):

$$M = \text{ATP Spike (nmole or pmole)} * DF * \left(\frac{(OD \text{ or } RFU_{Sample\ corrected})}{(OD \text{ or } RFU_{Spiked\ corrected}) - (OD \text{ or } RFU_{Sample\ corrected})}\right)$$

M= Amount of ATP in sample well (nmole or pmole)

ATP Spike (nmole or pmole) = quantity of ATP added (nmole or pmole) to the sample well.

DF = Dilution factor for the sample, applied if the sample is diluted to ensure it falls within the range of the standard curve before setting up the reaction wells.

OD/RFU sample corrected = OD/RFU of the sample after subtracting the sample blank and blank assay readings.

OD/RFU spike corrected = OD/RFU of the sample spike after subtracting the sample blank and blank assay readings.

Example for Sample Spike Calculation

OD Blank assay =0.046

OD Blank sample =1.188

OD sample=1.536

OD sample spike=1.799

ATP spike= 4nmole

Sample volume= 50 µl

DF=1

Concentration of ATP in sample:

OD sample corrected =

(1.536-0.046) -(1.188-0.046) = 0.348

OD spike corrected =

(1.799-0.046) -(1.188-0.046) = 0.611

Place in the equation:

$$M = 4_{(nmole)} * 1 * \left(\frac{(0.348)}{(0.611) - (0.348)}\right) = 5.3_{\left(\frac{nmole}{well}\right)}$$

For concentration, please divide by sample volume:

C = 5.3 nmole /50 µl = 0.106 nmole /µl

ATP molecular weight is 507.18 g/mole.

0.106 nmole /µl * 507.18 g/mole = 53.76 ng/µl

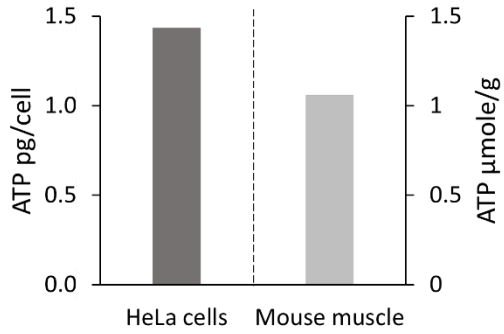

Figure 1.
Quantitation of ATP in mouse liver using the colorimetric assay and HeLa cells using the fluorometric assay.

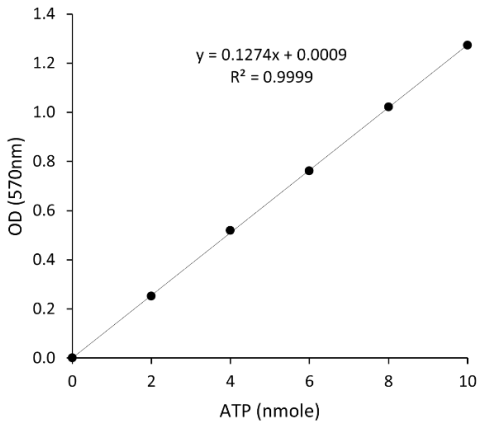

Figure 2.
ATP Calibration Curves of MAK603 Kit using the colorimetric method.

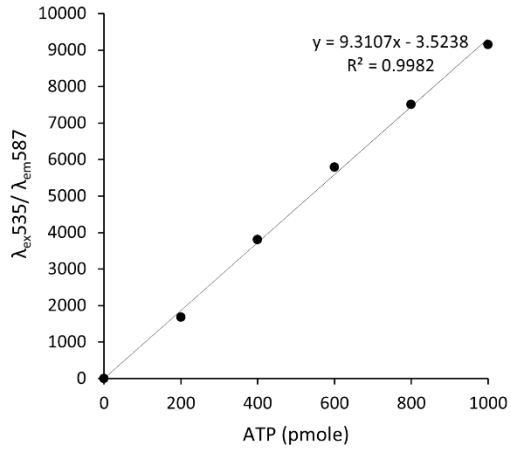

Figure 3. ATP Calibration Curves of MAK603 Kit using the fluorescent method.

References

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3. Carrozzo, R. et al. Infantile mitochondrial disorders. Biosci. Rep. 27, 105–112 (2007).

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mak603pis Rev 01/26