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Product Information

ATP Colorimetric/Fluorometric Assay Kit

Catalog Number **MAK190**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Adenosine triphosphate (ATP) is a nucleoside triphosphate formed exclusively in the mitochondria. It is a high-energy molecule described as the energy currency in all living systems. The chemical energy contained in the phosphate bond of ATP drives most cellular processes.[1,2] Genetic disorders such as Leber hereditary optic neuropathy,[2] Leigh syndrome, neuropathy, ataxia, and retinitis pigmentosa[3] affect the generation of ATP in the mitochondria.

The ATP Colorimetric/Fluorometric Assay Kit is a sensitive assay for determining ATP in various samples (ranging from 2–10 nmole/well for the colorimetric assay and from 20–1,000 pmole/well for the fluorometric assay). ATP concentration is determined by phosphorylating glycerol, resulting in a colorimetric (570 nm) or fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product proportional to the amount of ATP present. Note: The fluorometric assay is 10–100 times more sensitive than the colorimetric assay.

Components

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

ATP Assay Buffer Catalog Number MAK190A	25 mL
ATP Probe, in DMSO Catalog Number MAK190B	0.2 mL
ATP Converter Catalog Number MAK190C	1 vl
Developer Mix Catalog Number MAK190D	1 vl
ATP Standard, 1.0 μmole Catalog Number MAK190E	1 vl

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays and black plates for fluorometric assays.
- Spectrophotometric or fluorometric multiwell plate reader
- Liquid N_2 (optional for long-term sample storage)
- 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.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

ATP Assay Buffer – Store the buffer at –20 °C or 2–8 °C. Allow buffer to come to room temperature before use.

ATP Probe – Warm to room temperature prior to use to melt DMSO. Store at –20 °C, protected from light and moisture. Use within 2 months.

ATP Converter and Developer Mix – Reconstitute each with 220 μL of ATP Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C protected from light. Use within 2 months of reconstitution.

ATP Standard – Reconstitute with 100 μL of 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

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

ATP Standards for Colorimetric Detection
Dilute 10 µL of the 10 mM (10 nmole/µL) ATP Standard Solution with 90 µL of water to generate a 1 mM (1 nmole/µL) ATP Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM ATP Standard Solution into a 96 well plate generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add ATP Assay Buffer to each well to bring the volume to 50 µL.

ATP Standards for Fluorometric Detection
Dilute 10 µL of the 1 mM (1 nmole/µL) ATP Standard Solution with 90 µL of water to generate a 0.1 mM (100 pmole/µL) ATP Standard Solution or with 990 µL of water to generate a 0.01 mM (10 pmole/µL) ATP Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM ATP Standard Solution into a 96 well plate to generate 0 (blank), 200, 400, 600, 800, and 1,000 pmole/well standards or of the 0.01 mM ATP Standard Solution into a 96 well plate to generate 0 (blank), 20, 40, 60, 80, and 100 pmole/well standards. Add ATP Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Note: Sample preparation must be rapid to prevent the consumption of ATP by enzymes. Use of fresh samples is recommended since ATP is labile. Samples may be snap-frozen in liquid N_2 for later use.

Homogenize tissue (10 mg) or lyse cells (1×10^6) in 100 µL of ATP Assay Buffer. Deproteinize using a 10kDa MWCO spin filter.

To correct for background in samples, especially background caused by glycerol phosphate, include a Sample Blank by omitting the ATP Converter. The Sample Blank readings can then be subtracted from the sample readings.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Add 2–50 µL of samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with ATP Assay Buffer.

Spiking duplicate sample wells with a known amount of ATP Standard Solution is recommended for the accurate determination of ATP in samples that might contain endogenous compounds that interfere with the reaction.

Assay Reaction
1. Set up Reaction Mixes according to the schemes in Table 1 or Table 2. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Colorimetric Reaction Mixes

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

Table 2.
Fluorometric Reaction Mixes

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

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

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Incubate the plate at room temperature for 30 minutes. Protect the plate from light during the incubation.

3. Measure the absorbance at 570 nm (A_{570}) or the fluorescence (FLU, λ_{ex} = 535/ λ_{em} = 587 nm) in a microplate reader. The signals are stable for 2 hours.

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) ATP Standard. Correct for the background by subtracting the blank standard value from that of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the corrected values (A_{570} or FLU) obtained from the appropriate ATP Standards to plot a standard curve.

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

Subtract the Sample Blank value from the final sample reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of ATP present in the sample from the standard curve.

Concentration of ATP

$$C = S_a/S_v$$

where:

S_a = Amount of ATP in unknown sample well (nmole or pmole) from standard curve

S_v = Sample volume (μL) added into the well

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

ATP molecular weight is 507.17

Sample Calculation

Amount of ATP (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of ATP in sample:

$$5.84 \text{ nmole}/50\ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 507.17 \text{ ng/nmole} = 59.24 \text{ ng}/\mu\text{L}$$

Concentration of ATP in spiked samples

For spiked samples, calculate the amount of ATP in the sample wells after correcting for the Sample Blank and background.

$$C = \frac{S_p \times A_s}{(A_{sp} - A_s) \times S_v}$$

where:

S_p = Known amount of ATP Standard spiked in well (nmole or pmole)

A_s = Corrected sample reading (A_{570} or FLU) (unspiked well)

A_{sp} = Corrected sample + spike reading (A_{570} or FLU)

S_v = Sample volume (μL) added into the well

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

Sample Calculation

Amount of ATP Standard spike (S_p) = 300 pmole

Sample volume (S_v) = 50 μL

Corrected sample reading (A_s) = 4,599 (FLU)

Corrected spike + sample reading (A_{sp}) = 4,700 (FLU)

Concentration of ATP in sample:

$$\text{pmole}/\mu\text{L} = \frac{300 \text{ pmole spike} \times 4{,}599}{[(4{,}700 - 4{,}599) \times 50\ \mu\text{L}} = 273.2$$

References

1. Mitchell, P., Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature, **191**, 144–148 (1961).
2. Alirol, E., and Martinou, J.C., Mitochondria and cancer: is there a morphological connection? Oncogene, **25**, 4706–4716 (2006).
3. Carrozzo, R. et al., Infantile mitochondrial disorders. Biosci. Rep., **27**, 105–112 (2007).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates. For fluorescence assays, use black plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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