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Product Information

Adenylate Kinase Activity Assay Kit (Fluorometric and Colorimetric)

Catalog Number **MAK235**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Adenylate Kinase (AK, EC 2.7.4.3) is an abundant enzyme involved in energy metabolism and homeostasis of cellular adenine nucleotide ratios in different intracellular compartments. The enzyme is found in the nucleus, cytosol, or mitochondria (intermembrane space or matrix) of various kinds of tissues. Adenylate kinase acts on two molecules of ADP to generate ATP and AMP. Nine isoforms of adenylate kinase have been identified. Erythrocyte adenylate kinase deficiency is associated with hemolytic anemia. Adenylate kinase also plays an important role in post-ischemic recovery and in apoptosis.

This AK Activity Assay kit can kinetically measure Adenylate Kinase activity by detecting adenosine triphosphate (ATP) generated from adenosine diphosphate (ADP) as a substrate. ATP is detected via a multi-step reaction, resulting in the generation of an intermediate that reacts with the Adenylate Kinase Probe forming a colorimetric (570 nm)/fluorometric (λ_{ex} = 535 nm/λ_{em} = 587 nm) product.

$$2\ ADP \xrightarrow{\text{adenylate kinase}} ATP + AMP$$

$$ATP \xrightarrow{\text{convertor}} \xrightarrow{\text{developer}} Intermediate$$

$$Intermediate \xrightarrow{\text{adenylate kinase probe}} \text{end product}$$

end product - colorimetric (570 nm)/fluorometric (λ_{ex} = 535 nm/λ_{em} = 587 nm)

Components

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

AK Assay Buffer Catalog Number MAK235A	25 mL
AK Probe Catalog Number MAK235B	200 µL
ADP Substrate Catalog Number MAK235C	200 µL
AK Convertor Catalog Number MAK235D	1 vial
AK Developer Catalog Number MAK235E	1 vial
Positive Control (AK Enzyme) Catalog Number MAK235F	1 vial
ATP Standard (1 µmole) Catalog Number MAK235G	1 vial

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – white plates are recommended for this assay.
- Fluorescence or spectrophotometric multiwell plate reader
- Protease Inhibitor Cocktail

Precautions and Disclaimer

This product is 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 small vials at low speed prior to opening. Use ultrapure water for the preparation of reagents and standards.

AK Assay Buffer – Bring to room temperature before use. Store at –20 °C or 2–8 °C.

AK Convertor and AK Developer – Reconstitute each with 220 µL of AK Assay Buffer and mix gently by pipetting. Briefly centrifuge to collect the contents at the bottom of the tube. Aliquot and store at –20 °C. Avoid repeated freeze/thaw.

Positive Control (AK Enzyme) – Reconstitute with 55 µL of water. Store at –20 °C. Avoid repeated freeze/thaw cycles. Use within two months.

ATP Standard – Dissolve in 100 µL of water to generate a 10 mM stock solution. Keep on ice while in use. Store at –20 °C. Avoid repeated freeze/thaw cycles.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended. Briefly centrifuge all small vials prior to opening.

Procedure
All samples and standards should be run in duplicate. Read entire protocol before performing the assay.

Sample Preparation

Tissue – Rinse tissue and transfer ~50 mg of fresh or frozen tissue (stored at –80 °C) to a prechilled tube. Add 150 µL of cold AK Assay Buffer containing a protease inhibitor cocktail (not provided) and thoroughly homogenize tissue on ice using an electrical homogenizer. Transfer the tissue homogenate to a cold microfuge tube.

Cells – To prepare cell extract, add 150 µL of cold Homogenization Buffer containing a protease inhibitor cocktail (not provided) to 1–5 × 10^6 fresh or frozen cells and pipette several times to disrupt the cells. Transfer cell homogenate including cell debris to a cold microfuge tube and agitate on a rotary shaker at 4 °C for at least 15 minutes. Centrifuge the tissue or cell homogenate at 16,000 × *g* at 4 °C for 10 minutes. Transfer the clarified supernatant to a fresh pre-chilled tube and store on ice. Use lysates immediately to assay Adenylate Kinase activity. Mitochondria can be isolated using a Mitochondria Isolation Kit and solubilized in AK Assay Buffer for 10 minutes on ice prior to use.

Add 2–50 µL of cell/tissue homogenate, mitochondrial lysate, or purified protein into 96 well plate. For colorimetric assay, use 2–5 µL of Positive Control. For fluorometric assay, dilute Positive Control 5-fold with AK Assay Buffer just before use. Add 2–5 µL of diluted Positive Control for the assay. Bring the volume of samples and Positive Control to 50 µL/well with AK Assay Buffer. Add 50 µL AK Assay Buffer to one well as reagent background control.

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

ATP and glycerol-3-phosphate in the samples will contribute to the background. Prepare parallel sample well(s) as sample background control(s) and adjust the volume to 50 µL.

Lysates can be aliquoted and snap frozen in liquid nitrogen before storing at –20 °C. Avoid freeze/thaw cycles.

ATP Standard

For the colorimetric assay, dilute 10 µL of the ATP Standard with 90 µL of water to generate 1 mM ATP Standard, mix well. Add 0, 2, 4, 6, 8, and 10 µL of 1 mM ATP Standard into a series of wells in a 96 well plate and adjust the volume to 50 µL/well with AK Assay Buffer to generate 0, 2, 4, 6, 8, and 10 nmole/well of ATP Standard. For the fluorometric assay, further dilute the ATP Standard to 0.1 mM with water (detection sensitivity is 10 to 100-fold higher with the fluorometric than with the colorimetric assay). Follow the procedure as for the colorimetric assay to give 0, 0.2, 0.4, 0.6, 0.8 and 1 nmole/well of ATP Standard.

Reaction Mixes
Set up appropriate mixes according to the scheme in Table 1. 50 µL of the appropriate mix is required for each reaction (well).

Table 1.
Preparation of Mixes

	Reaction Mix	Background Control Mix
AK Assay Buffer	42.5 µL	44.5 µL
AK Convertor	2 µL	2 µL
AK Developer	2 µL	2 µL
ADP Substrate	2 µL	–
AK Probe*	1.5 µL	1.5 µL

Add 50 µL of Reaction Mix to wells for each sample, reagent background control, and Positive Control, and 50 µL of Background Control mix to wells for Standards and Sample Background Control. Mix well.

Measurement
Pre-incubate for five minutes at room temperature and measure absorbance (570 nm) or fluorescence (λ_{ex} = 535 nm/λ_{em} = 587 nm) in kinetic mode for 30–60 minutes at room temperature. Choose two time points (T_1 & T_2) in linear range (can be as short as 2 minutes) of plot and obtain corresponding absorbance or fluorescence for sample (R_{S1} and R_{S2}) and reagent background control (R_{BG1} and R_{BG2}). Read the ATP Standard Curve along with the samples.

Results
Calculations
Subtract 0 Standard reading from all Standard Readings. Plot the ATP Standard Curve.

Subtract reagent background control reading from sample readings. Apply the ΔR [($R_{S2} – R_{BG2}$) – ($R_{S1} – R_{BG1}$)] to the Standard Curve to get B nmole of ATP generated by the sample during the reaction time ($\Delta T = T_2 – T_1$).

Note
If sample background control reading is significant, subtract sample background control reading from sample reading instead of subtracting reagent background control reading and use this ΔR to determine B nmole of ATP generated by the sample during the reaction time ($\Delta T = T_2 – T_1$).

$$\text{Adenylate Kinase Activity (nmole/min/µg)} = \frac{B}{\Delta T \times \mu g \text{ of protein}}$$

B = ATP amount from Standard Curve (nmole).
ΔT = the reaction time (min.)
µg of protein = the amount of protein/well (µg)

Adenylate Kinase Activity can also be expressed as mU/mg (nmole/min ATP generated per mg) of protein.

Unit Definition: One unit of Adenylate Kinase activity is the amount of enzyme that generates 1.0 µmole of ATP/minute under the assay conditions.

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	White plates are recommended for this assay
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 samples will be used 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 Mix 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 mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix 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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