Adenylate Kinase (AK) Activity Assay Kit (Colorimetric/Fluorometric)

2/17

(Catalog # K350-100; 100 assays; Store at -20°C)

I. Introduction:

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. BioVision's 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 an end product that can be measured colorimetrically (OD 570 nm) or fluorometrically (Ex/Em = 535/587 nm).

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

$$ATP \xrightarrow{\text{Convertor}} \xrightarrow{\text{Developer}} \text{Intermediate + Adenylate Kinase Probe} \longrightarrow \text{End Product (Ex/Em = 535/587 nm or OD 570 nm)}$$

II. Application:

- Detection of Adenylate Kinase activity

III. Sample Type:

- Purified Adenylate Kinase
- Cell and tissue lysate
- Mitochondrial lysate

IV. Kit Contents:

Components	K350-100	Cap Code	Part Number
AK Assay Buffer	25 ml	WM	K350-100-1
AK Probe	200 µl	Red	K350-100-2
ADP Substrate	200 µl	Brown	K350-100-3
AK Convertor	1 vial	Blue	K350-100-4
AK Developer	1 vial	Green	K350-100-5
Positive Control (AK Enzyme)	1 vial	Clear	K350-100-6
ATP Standard (1 µmol)	1 vial	Yellow	K350-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear (colorimetric) or black plate (fluorometric) with flat bottom.
- Microplate reader capable of absorbance or fluorescence detection
- Protease Inhibitor Cocktail (Cat. # K271 or equivalent)

VI. Storage Conditions and Reagent Preparation:-

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **AK Assay Buffer:** Bring to room temperature before use. Store at -20°C or 4°C.
- **AK Convertor and AK Developer:** Reconstitute each with 220 µl 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 deionized water. Store at -20°C. Avoid repeated freeze/thaw. Use within two months.
- **ATP Standard**: Dissolve in 100 µl of distilled water to generate a 10 mM stock solution. Keep on ice while in use. Store at -20°C. Avoid repeated freeze/thaw.

VII. Adenylate Kinase Activity Assay Protocol:

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

   To prepare cell extract, add 150 µl cold Homogenization Buffer containing protease inhibitor cocktail (not provided) to 1-5 x 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 min. Centrifuge the tissue or cell homogenate at 16,000 X g, 4°C for 10 min. Transfer the clarified supernatant to a fresh pre-chilled tube & store on ice. Use lysates immediately to assay Adenylate Kinase activity. Mitochondria can be isolated using BioVision's Mitochondria Isolation Kit (K288) and solubilized in AK Assay Buffer for 10 min. 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 Positive Control. For fluorometric assay, dilute Positive Control 5x in AK Assay Buffer just before use. Add 2-5 µl of diluted Positive Control for the assay. Make up 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:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

b. 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.

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

2. **ATP Standard:** For the colorimetric assay, dilute 10 µl of the ATP Standard with 90 µl of dH_2O 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 nmol/well of ATP Standard. For the fluorometric assay, further dilute the ATP Standard to 0.1 mM with dH_2O (detection sensitivity is 10-100 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 nmol ATP Standard.

3. **Reaction Mix:** Prepare enough reagents for the number of assays to be performed. Make 50 µl of Reaction Mix and Background Control Mix containing:

	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 into each Sample, reagent background control and Positive Control wells and 50 µl of Background Control mix to Standards and Sample Background Control well(s). Mix well.

*For fluorometric assays, reduce the amount of probe and add 0.3 µl per reaction mix and background control mix.

4. **Measurement:** Pre-incubate for five min. at room temperature and measure absorbance (570 nm) or fluorescence (Ex/Em = 535/587 nm) in kinetic mode for at least 30-60 min. at room temperature. Choose two time points (T_1 & T_2) in linear range (can be as short as 2 min.) 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.

5. **Calculations:** Subtract 0 Standard reading from all Standard Readings. Plot the ATP Standard Curve. Subtract reagent background control reading from sample reading. Apply the ΔR $[(R_{S2}- R_{BG2}) – (R_{S1}- R_{BG1})]$ to the Standard Curve to get B nmol of ATP generated by the sample during the reaction time (ΔT = T2 - T1).

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 nmol of ATP generated by the sample during the reaction time (ΔT = T2 - T1).

$$\textbf{Sample's Adenylate Kinase Activity} = \frac{\mathbf{B}}{\Delta T \times \mu g\ of\ protein} = \textbf{nmol/min/µg = mU/µg}$$

Where: **B** is ATP amount from Standard Curve (nmol).
Δ**T** is the reaction time (min.)
µg of protein is the amount of protein/well in µg

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

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

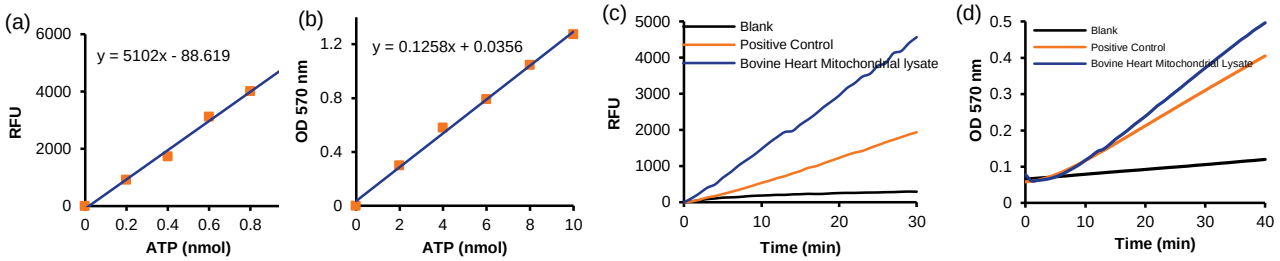

Figure: (a) ATP Standard Curve (fluorometric), (b) ATP Standard Curve (colorimetric), (c) Fluorometric quantitation of Adenylate Kinase Activity in bovine heart mitochondrial lysate (50 ng) and Positive Control (4 µl); (d) Colorimetric quantitation of Adenylate Kinase Activity in bovine heart mitochondrial lysate (500 ng) and Positive Control (5 µl).

VIII. RELATED PRODUCTS:

Xanthine Oxidase Colorimetric/Fluorometric Assay Kit (K710)
Xanthine/Hypoxanthine Colorimetric/Fluorometric Assay Kit (K685)
ADP Colorimetric/Fluorometric Assay Kit (K355)
ATP Colorimetric/Fluorometric Assay Kit (K354)
Adenosine Deaminase Assay Kit (Colorimetric) (K321)
Bioluminescence Cytotoxicity Assay Kit (K312)
PNP Activity Assay Kit (Fluorometric) (K767)
Inosine Fluorometric Assay Kit (K712)
Uric Acid Colorimetric/Fluorometric Assay Kit (K608)
ADP Colorimetric Assay Kit II (K356)
Adenosine Deaminase Assay Kit (Fluorometric) (K328)
Adenosine Assay Kit (Fluorometric) (K327)
Mitochondria Isolation Kit for Tissue & Cultured Cells (K288)
PNP Activity Assay Kit (Colorimetric) (K768)

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