Adenylate Kinase Activity Assay Kit

Cat. No. Kit-2147 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This ADK Activity Assay Kit provides a sensitive and robust method to quantify AK activity by coupling the enzymatic reaction to NADPH oxidation, which is monitored spectrophotometrically at 340 nm. The assay employs a two-step enzymatic cascade: 2ADP ➔ ATP + AMP ATP + Glucose ➔ G6P + ADP G6P + NADP+ ➔ 6-Phosphgluconate + NADPH + H+ The oxidation of NADPH to NADP (absorbance decrease at 340 nm) is proportional to ADK activity.
Description	Adenylate Kinase (ADK, EC 2.7.4.3) is a highly conserved enzyme that plays a pivotal role in cellular energy metabolism by maintaining adenine nucleotide homeostasis. It is ubiquitously expressed in the nucleus, cytosol, and mitochondria of eukaryotic cells, where it catalyzes the reversible transfer of a phosphate group between adenine nucleotides: 2ADP➔ATP+AMP This reaction is critical for energy buffering, nucleotide recycling, and cellular signaling. Dysregulation of AK activity has been implicated in various pathological conditions, including hemolytic anemia and ischemiareperfusion injury.
Components	The kit is sufficient for 5 00 assays in 96 well plates. ADK Assay Regent 1: 140 mL ADK Assay Regent 2: 35 mL ATP Standard (2 mM): 5 mL 5 vial ADK Positive control (130-150 U/L): 5 vial
Reagents and Equipment Required but Not Provided	1. 96 well flat-bottom plate: white plates are recommended for this assay. 2. Spectrophotometric multiwell plate reader. 3. Protease Inhibitor Cocktail.
Precautions and	This product is for R&D use only, not for drug, household, or other uses. Please

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Disclaimer	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. ADK Assay Regent 1&2: Bring to room temperature before use. Store at 2–8 centigrade. ATP Standard: Keep on ice while in use. Store at –20 centigrade. Avoid repeated freeze/thaw cycles. Use within two months. Positive Control (ADK Enzyme): Reconstitute with 250 µL of water. Store at –20 centigrade. Avoid repeated freeze/thaw cycles.
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 centigrade) 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 centigrade for at least 15 minutes. Centrifuge the tissue or cell homogenate at 16,000×g at 4 centigradefor 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

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	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 centigrade. Avoid freeze/thaw cycles. ATP Standard For the colorimetric assay, dilute 50 µL of the ATP Standard with 50 µL of water to generate 1 mM ATPStandard, 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 240 µL/well to generate 0, 2, 4, 8, and 10 nmole/well of ATP Standard. 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. 150 µL of the appropriate mix is required for each reaction (well). Add 150 µL of Reaction Mix to wells for each sample, reagent background control, and Positive Control, and 150 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 (340 nm) in two-point end mode for 5-10 minutes at 37 centigrade. Choose two time points (T1 & T2) in linear range (Can be as short as 2 minutes) of plot and obtain corresponding absorbance 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.
Result	Calculations Subtract 0 Standard reading from all Standard Readings. Plot the ATP Standard Curve. Subtract reagent background control reading fromsample readings. Apply the ΔR [(R s2-R BG2)-(R s1-R Bci)] to the Standard Curve to get B nmole of ATPgenerated by the sample during the reaction time(ΔT=T2-T1). Note: If sample background control reading is significant, subtract sample background control reading fromsample reading instead of subtracting reagentbackground control reading and use this ΔR todetermine B nmole of ATP generated by the sampleduring the reaction

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	time (ΔT=T2-T1). Adenylate Kinase Activity (nmole/min/ug) =B/(ΔT x ug 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 isthe amount of enzyme that generates 1.0 umole of ATP/minute under the assay conditions.
Storage	The kit is shipped on wet ice and storage at 2-8 centigrade, protected from light is recommended. Briefly centrifuge all small vials prior to opening.
Table 1	**Table 1.** Preparation of Mixes Reaction Mix / Background Control Mix ADK Assay R1: 120 µL / 120 µL ADK Assay R2: 30 µL / 30 µL

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