

BIOMEDICAL RESEARCH SERVICE CENTER

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Adenylate Kinase (ADK) Assay Kit (Cat#: E-121)

COMPONENTS: ADK Reaction Solution- 0.5 ml, store at -80°C
ATP Assay Solution- 10 ml (100 assays), store in aliquots shielded from light at -80°C
4 mM EDTA- 10 ml, store at 4°C
10x Cell Lysis Solution- 25 ml, store at 4°C (contains 1% TX-100; swirl bottle briefly prior to dilution)

PRODUCT DESCRIPTION: Chemiluminescent detection of ADK-mediated formation of ATP allows fast and sensitive measurement of ADK activity present in tissue/cell extracts and biological fluids such as serum/plasma. Kit components are stable for several years if stored and handled properly. The assay requires the use of a luminometer.

ATP Assay Solution: ATP Assay Solution should be stored at -80°C in aliquots after its first thawing. It should be shielded from light during assay.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH₂O. Bring up at least ~10⁵ washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80°C.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.1 – 0.5 mg/ml.

Assay Protocol:

1. **Setup-** Keep all thawed reagents on ice. Set up two sets of 0.5-ml microtubes for Control and Reaction for each sample to be assayed. Add 5 µl dH₂O to each tube of the Control set and 5 µl ADK Reaction Solution to each tube of the Reaction set. Proceed to steps 2 – 3 to initiate PK reaction.
2. **Control set-** Add 5 µl of the first sample to 5 µl dH₂O and mix by pipetting up and down swiftly. Allow reaction to proceed for 1 min at room temperature. Terminate reaction by adding 90 µl 4 mM EDTA followed by brief vortexing. Transfer tube to ice.
3. **Reaction set-** Add 5 µl of the first sample to 5 µl ADK Reaction Solution and mix by pipetting up and down swiftly. Allow reaction to proceed for 1 min at room temperature. Terminate reaction by adding 90 µl 4 mM EDTA followed by brief vortexing. Transfer tube to ice. Repeat steps 2 - 3 for the next sample.

RLU Measurement:

1. Thaw enough ATP Assay Solution and warm to room temperature shielded from light. Blank: add 10 µl of dH₂O to a luminometer tube or well. Control and Reaction sets: add 10 µl of Control (step 2) and Reaction (step 3) of each sample to the respective luminometer tubes/wells.
2. Gently vortex thawed ATP Assay solution prior to pipetting. Use 0.1 ml ATP Assay Solution for Blank, each Control, and each Reaction tube/well. Immediately measure the relative light unit (RLU) using a luminometer.
3. Subtract Blank RLU from all Control and Reaction RLU readings. Then subtract Control RLU from Reaction RLU for each sample.
4. Sample ADK activity is expressed as RLU/µg protein (for cell/tissue lysate) and RLU/µl (for plasma/serum).