BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: ADK Substrate- 0.25 ml, store at -80ºC
ATP Assay Solution- 10 ml (for 100 assays), store at -80ºC **(shield solution from light during use)**
TCA Stop- 1 ml, store at 4ºC (**caution: avoid contact with skin**)
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents upon arrival)**

PRODUCT DESCRIPTION: Chemiluminescent detection of adenylate kinase (ADK)-mediated formation of ATP provides a sensitive assay of ADK activity present in tissue/cell extracts, serum, and cell culture medium. ADK activity is determined by measurement of Relative Light Unit (RLU) using luminometer. Kit components are stable for at least one year if stored and handled properly.

Preparation of cell/tissue and serum samples:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up ~10^5 freeze-thawed cells in ~100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. Fresh or freeze-thawed tissue is homogenized in ice-cold 1x Cell Lysis Solution (~2 mg tissue in 0.5 ml).

2. Centrifuge lysate in a refrigerated microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and kept on ice. Use the BCA protein assay method to determine protein concentration. Adjust sample protein concentration to 0.05 - 0.5 mg/ml using ice-cold 1x Cell Lysis Solution as diluent. *Normalization of sample protein concentration is recommended.* Store samples at -80ºC.

3. Serum samples should be diluted 3-fold with ice-cold dH_2O for the assay. *Do not use plasma or undiluted serum for the assay.*

Enzyme assay:

1. Keep thawed ADK Substrate and ATP Assay Solution on ice. *Do not over thaw*. ATP Assay Solution should be shielded from light during assay.

2. Add 2.5 µl ADK Substrate to each of a set of 0.5-ml microtubes and keep on ice.

3. For assay control, add 2.5 µl 1x Cell Lysis Solution and 5 µl TCA Stop to a designated tube containing 2.5 µl ADK Substrate. Mix contents and place the control tube on ice.

4. Proceed to analyze the first sample by adding 2.5 µl lysate to 2.5 µl ADK Substrate and mix by pipetting up and down twice. Immediately place tube in a 37°C-water bath for exactly 1 min (for cell/tissue lysate) or 5 min (for cell culture medium/diluted serum). Remove tube from water bath and immediately terminate reaction by adding 5 µl TCA Stop. Vortex tube and place tube on ice. *Reaction time can be optimized by end users.*

5. Repeat Step 4 for the next sample and so on. Keep reaction time the same for all samples. All sample tubes should be kept on ice for at least 10 min after addition of TCA Stop.

6. Centrifuge control and sample tubes in a microfuge for 5 min (~14,000 rpm).

7. Remove tubes from centrifuge. Add 0.25 ml ice-cold dH_2O to each tube. Mix solution and centrifuge for 3 min.

8. Transfer 10 µl of supernatant from each tube to a clear (uncoated) 96-well plate for measurement of Relative Light Unit (RLU) using a luminometer. Add 0.1 ml ATP Assay Solution to each well and record RLU.

9. Subtract control RLU from sample RLU to obtain **ΔRLU** for each sample. ADK activity is represented by **ΔRLU**, which can be normalized by protein concentration or GAPDH enzyme activity of each sample. *Increase sample protein concentration or reaction time at Step 4 if low ΔRLU is obtained.*

Additional information:

- Please visit the product webpage or contact us for material SDS information.
- 10x Cell Lysis Solution precipitates upon freezing. Re-dissolve contents by agitation in warm water upon arrival.