BIOMEDICAL RESEARCH SERVICE CENTER
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Nucleoside Diphosphate Kinase (NDK) Assay Kit (Cat #: E-129)

COMPONENTS: NDK Reaction Solution- 0.4 ml, store at -80ºC
NDK Control Solution- 0.4 ml, store at -80ºC
ATP Assay Solution- 10 ml (for 100 assays), **store in aliquots shielded from light at -80ºC**
4 mM EDTA- 10 ml, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**

PRODUCT DESCRIPTION: The NDK activity assay kit is based on chemiluminescent detection of NDK-mediated formation of ATP, which allows fast and sensitive measurement of NDK 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 dH_2O. Bring up at least ~10^5 washed cells in 100 – 200 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 -80ºC.
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.

Enzyme assay protocol:

1. **Setup**- Keep 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 NDK Control Solution to each tube of the Control set and 5 µl NDK Reaction Solution to each tube of the Reaction set. Keep tubes at room temperature for ~5 min. Proceed to steps 2 − 3.
2. **Control set**- Add 5 µl of the first sample to 5 µl NDK Control Solution. Mix contents by swiftly pipetting up and down 10 times. Allow reaction to proceed for 1 min at room temperature. The assay measures background activity. Terminate reaction by adding 90 µl EDTA solution followed by brief vortexing. Transfer tube to ice.
3. **Reaction set**- Add 5 µl of the first sample to 5 µl NDK Reaction Solution. Mix contents by pipetting up and down 10 times. Allow reaction to proceed for 1 min at room temperature. Terminate reaction by adding 90 µl EDTA solution 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 dH_2O to a luminometer tube or well. Control and Reaction sets: add 10 µl from Control (step 2) and Reaction (step 3) of each sample to the respective luminometer tubes/wells.
2. 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 NDK activity is expressed as RLU/µg protein (for cell/tissue lysate) and RLU/µl (for plasma/serum).