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Nucleoside Diphosphate Kinase (NDK or NDPK) Assay Kit (Cat #: E-129)

COMPONENTS: NDK Control- 0.25 ml, store at -80ºC
NDK Reaction- 0.25 ml, store at -80ºC
ATP Assay Solution- 2x10 ml (for 100 assays), store at -80ºC **(shield solution from light during use)**
TCA Stop- 1 ml, store at 4ºC (**caution: avoid skin contact**)
10x Sample Buffer- 25 ml, store at 4ºC

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

Preparation of cell/tissue and serum samples:

1. PBS-washed cell/tissue samples should be frozen at -80ºC prior to homogenization. At least ~10^5 cells or 2 mg of tissue should be used for lysate preparation. Freeze thawing, mechanical grinding, or sonication facilitates protein extraction.
2. Prepare 1x Sample Buffer by diluting 10x Sample Buffer with dH_2O. Bring up freeze-thawed cells in 50–100 µl ice-cold 1x Sample Buffer by pipetting up and down. Adherent cells can be directly harvested from plate by scraping. Leave lysate on ice for 5 min with agitation. Freeze-thawed tissue is mechanically homogenized in ice-cold 1x Sample Buffer (~5 mg tissue in 0.5 ml).
3. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC. Perform protein assay to determine sample protein concentration. Normalize sample protein concentration to 0.1 – 0.5 mg/ml.
4. Serum samples should be diluted 3-fold with ice-cold dH_2O prior to assay. *Do not use plasma or undiluted serum for the assay.*

Enzyme assay:

1. Keep thawed NDK Control, NDK Reaction, and ATP Assay Solution on ice. *Do not over thaw*. Shield ATP Assay Solution from light during assay.
2. Pipette 2.5 µl NDK Control to one set of 0.5-ml microtubes and 2.5 µl NDK Reaction to another set of 0.5-ml microtubes. Keep tubes on ice.
3. Initiate assay by adding 2.5 µl of the first sample to NDK Control and mix solution 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/serum). Remove tube from water bath and immediately add 5 µl TCA Stop. Vortex tube and place tube on ice. *Reaction time may be optimized by end users.*
4. Proceed to add 2.5 µl of the first sample to NDK Reaction and mix solution 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/serum). Remove tube from water bath and immediately add 5 µl TCA Stop. Vortex tube and place tube on ice.
5. Follow Steps 3 - 4 for the next sample. 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 at ~14,000 rpm for 5 min.
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. NDK activity is represented by **ΔRLU**, which can be normalized by protein concentration or GAPDH enzyme activity of each sample.

Additional information:

- Please visit the product webpage or contact us for material SDS information.