NAD Kinase Microplate Assay Kit

User Manual

Catalog # FTA0040

(Version 1.1C)

Detection and Quantification of NAD Kinase (NADK) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

NAD kinase catalyzes the transfer of a phosphate group from ATP to NAD^+ to generate $NADP^+$, which in its reduced form acts as an electron donor for biosynthetic reactions. $NADP^+$ is an essential coenzyme in metabolism and provides reducing power to biosynthetic processes such as fatty acid biosynthesis.

The assay is initiated with the enzymatic hydrolysis of the NAD^+ by NADK. The enzyme catalysed reaction products can be measured at a colorimetric readout at 600 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate Diluent	16 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	-20 °C, keep in dark
Stop Solution	20 ml x 1	4 °C
Dissolution Buffer	30 ml x 1	4 °C
Standard	Powder x 1	-20 °C, keep in dark
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Note:

Substrate: add 8 ml Substrate Diluent to dissolve before use.

Enzyme: add 10 ml distilled water to dissolve before use, mix, store at 4°C.

Dye Reagent: add 1 ml distilled water to dissolve before use, mix, store at 4°C.

Standard: add 1 ml distilled water to dissolve, mix; then add 25 µl solution into 975 µl distilled water, mix. The concentration will be 50 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Sample	20 µl	--	--	--
Assay Buffer	--	20 µl	--	--
Substrate	80 µl	80 µl	--	--
Mix, put it in the oven, 37 °C for 10 minutes. Then put it in boiling water for 2 minutes. When cold, centrifuged at 10000g, room temperature for 10 minutes, take the supernatant into the new microcentrifuge tubes.				
The supernatant	20 µl	20 µl	--	--
Standard	--	--	20 µl	--
Distilled water	--	--	--	20 µl
Enzyme	90 µl	90 µl	90 µl	90 µl
Dye Reagent	10 µl	10 µl	10 µl	10 µl
Mix, keep them in dark for 2 minutes at room temperature.				
Stop Solution	200 µl	200 µl	200 µl	200 µl
Mix, stand at room temperature for 1 minutes, centrifuged at 20,000g for 5 minutes, discard the supernatant after centrifugation.				
Dissolution Buffer	300 µl	300 µl	300 µl	300 µl
Add 200 µl solution into the microplate, record absorbance measured at 570 nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

VI. CALCULATION

Unit Definition: One unit of NADK activity is the enzyme that generates 1 nmol of NADP per minute.

1. According to the protein concentration of sample

$$NADK\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T \times 10$$

$$= 50 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$NADK\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 50 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$NADK\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 50 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the standard concentration, 50 μmol/L = 50 nmol/ml

W: the weight of sample, g;

V_{Sample}: the volume of sample, 0.02 ml;

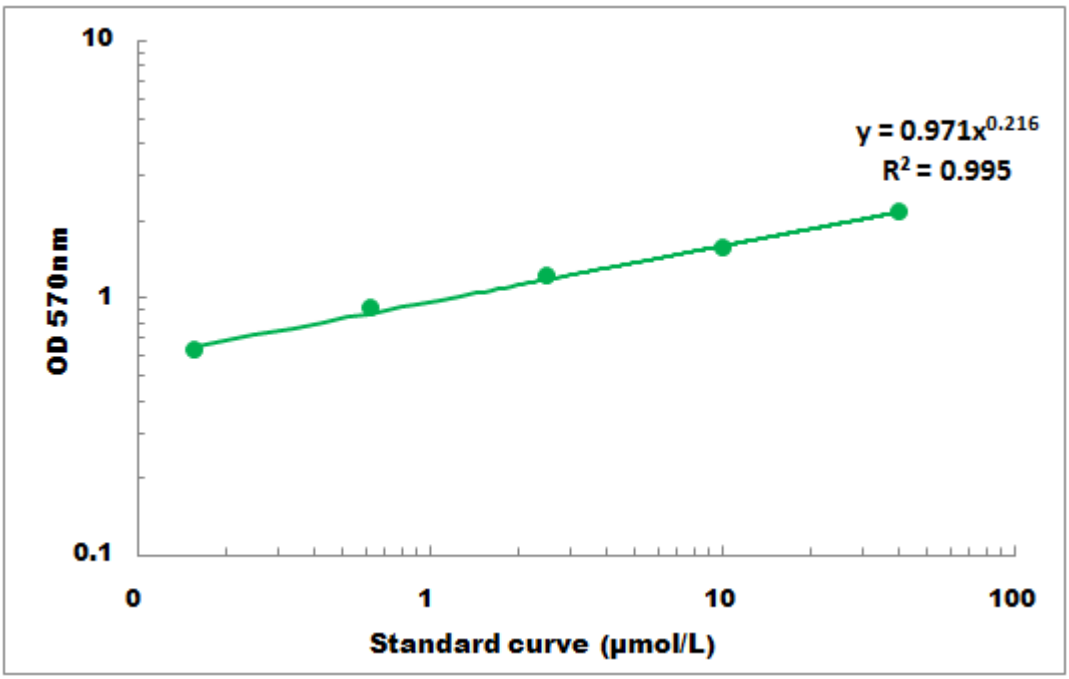
V_{Assay}: the volume of Assay buffer, 1 ml;

N: the quantity of cell or bacteria, N × 10^4;

T: the reaction time, 10 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 μmol/L - 50 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES