NAD/NADH Microplate Assay Kit

User Manual

Catalog # ASK1008

Detection and Quantification of NAD/NADH Content 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.

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I. INTRODUCTION……………………………………………………………………………………2

II. KIT COMPONENTS………………………………………………………………………………3

III. MATERIALS REQUIRED BUT NOT PROVIDED……………………………………………4

IV. SAMPLE PREPARATION………………………………………………………………………5

V. ASSAY PROCEDURE……………………………………………………………………………7

VI. CALCULATION……………………………………………………………………………………8

VII. TYPICAL DATA…………………………………………………………………………………10

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I. INTRODUCTION

Nicotinamide adenine dinucleotide (NAD^+) is a vital coenzyme found in all cells. As NAD^+ is involved in redox reactions, it is found in two forms in cells. NAD^+ is an oxidizing agent and becomes reduced to form NADH, which can be used as a reducing agent. As a result, it plays a key role in metabolism and other cellular processes. In organisms, NAD^+ can be synthesized de novo from tryptophan or aspartic acid. Because of the wide variety of functions that NAD^+ plays, it is a popular target for pharmaceuticals.

NAD/NADH Microplate Assay Kit is based on a glucose dehydrogenase cycling reaction, in which the formed NADH reduces a formazan (MTT) reagent. The intensity of the reduced product color, measured at 570 nm, is proportionate to the NAD^+/NADH concentration in the sample.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 2	4 °C
Assay Buffer II	30 ml x 2	4 °C
Dye Reagent	Powder x 1	-20 °C, keep in dark
Enzyme	Powder x 1	-20 °C
Substrate	10 ml x 1	4 °C
Stop Solution	20 ml x 1	4 °C
Dissolution Buffer	30 ml x 1	4 °C
NADH Standard	Powder x 1	-20 °C, keep in dark
NAD^+ Standard	Powder x 1	-20 °C, keep in dark
Technical Manual	1 Manual	

Note:

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

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

NADH 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.

NAD^+ 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.

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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

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IV. SAMPLE PREPARATION

1. For serum or plasma samples

Extract the NAD^+:

Add 0.5 ml Assay buffer I to 0.05 ml serum or plasma; mix; boiling for 5 minutes; centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 0.5 ml Assay buffer II, mix; keep it on ice for detection.

Extract the NADH:

Add 0.5 ml Assay buffer II to 0.05 ml serum or plasma; mix; boiling for 5 minutes; centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 0.5 ml Assay buffer I, mix; keep it on ice for detection.

2. For tissue samples

Extract the NAD^+:

Weigh out 0.05g tissue, homogenize with 0.5 ml Assay buffer I on ice; boiling for 5 minutes; centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 0.5 ml Assay buffer II, mix; keep it on ice for detection.

Extract the NADH:

Weigh out 0.05g tissue, homogenize with 0.5 ml Assay buffer II on ice; boiling for 5 minutes; centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 0.5 ml Assay buffer I, mix; keep it on ice for detection.

3. For cell and bacteria samples

Extract the NAD^+:

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5 ml Assay buffer I for 250×10^4 cell or bacteria, sonicate (with power 20%, sonication 2s, intervation 1s, repeat 30 times); boiling for 5 minutes;

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centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 0.5 ml Assay buffer II, mix; keep it on ice for detection.

Extract the NADH:

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5 ml Assay buffer II for 250 × 10^4 cell or bacteria, sonicate (with power 20%, sonication 2s, intervation 1s, repeat 30 times); boiling for 5 minutes; centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 0.5 ml Assay buffer I, mix; keep it on ice for detection.

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V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	30 µl
Substrate	80 µl	80 µl	80 µl
Dye Reagent	10 µl	10 µl	10 µl
Stop Solution	--	--	--
Enzyme	10 µl	10 µl	--
Mix, keep them in dark for 2 minutes at room temperature.			
Stop Solution	200 µl	200 µl	200 µl
Mix, stay 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
Add 200 µl solution into the microplate, record absorbance measured at 570 nm.			

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VI. CALCULATION

Calculation of NAD^+:

1. According to the volume of sample

NAD^+ (μmol/ml) = $C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Sample} / (V_{Sample} / V_{Assay})$

$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

2. According to the protein concentration of sample

NAD^+ (μmol/mg) = $C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Sample} / (V_{Sample} \times C_{Protein})$

$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

3. According to the weight of sample

NAD^+ (μmol/g) = $C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Sample} / (W \times V_{Sample} / V_{Assay})$

$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

4. According to the quantity of cells or bacteria

NAD^+ (μmol/10^4) = $C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Sample} / (N \times V_{Sample} / V_{Assay})$

$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

Calculation of NADH:

1. According to the volume of serum or plasma

NADH (μmol/ml) = $C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Sample} / (V_{Sample} / V_{Assay})$

$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

2. According to the protein concentration of sample

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NADH (µmol/mg) = $C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Sample} / (V_{Sample} \times C_{Protein})$

$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

3. According to the weight of sample

NADH (µmol/g) = $C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Sample} / (W \times V_{Sample} / V_{Assay})$

$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

4. According to the quantity of cells or bacteria

NADH (µmol/10^4) = $C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Sample} / (N \times V_{Sample}/V_{Assay})$

$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

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

$C_{Standard}$: the protein concentration, 50 µmol/L = 0.05 µmol/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, $N \times 10^4$.

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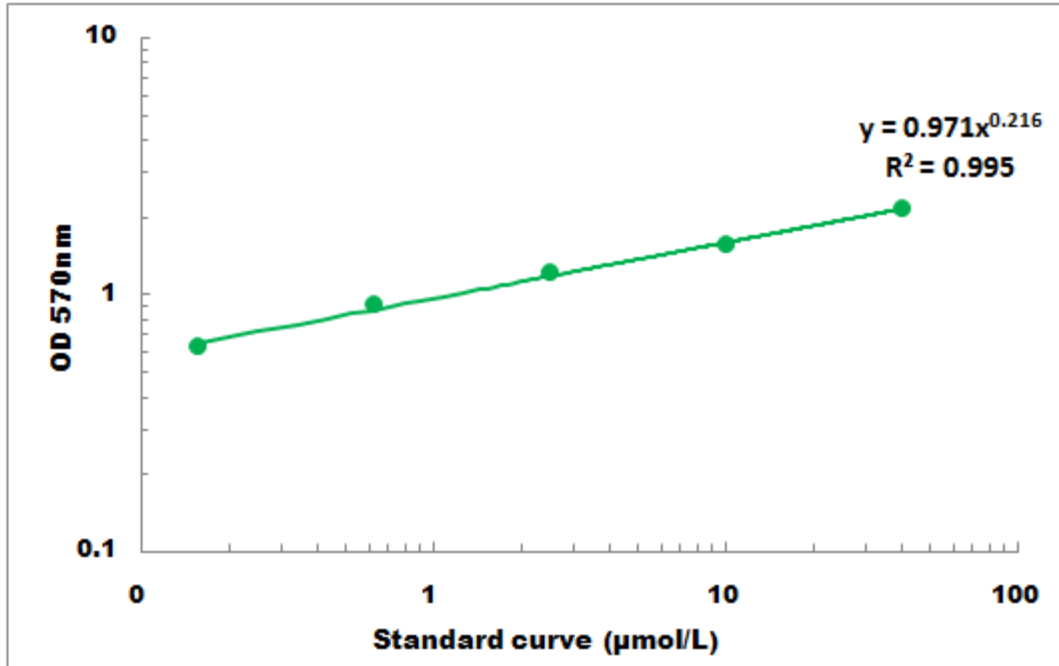
VII. TYPICAL DATA

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

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