NAD/NADH Microplate Assay Kit

User Manual

Catalog # FTA0008

(Version 2.1D)

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.

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 provides a simple and direct procedure for measuring NAD^+/NADH levels in a variety of samples. The kit is based on an alcohol dehydrogenase cycling reaction, in which the formed NADH reduces a formazan reagent. The intensity of the reduced product color, measured at 492 nm, is proportionate to the NAD^+/NADH concentration in the sample.

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
Reaction Buffer	10 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Substrate	10 ml x 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 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
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Note:

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

Enzyme: add 1 ml Reaction Buffer 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.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 492 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

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; 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.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Reaction Buffer	70 µl	70 µl	70 µl
Enzyme	10 µl	10 µl	10 µl
Substrate	80 µl	80 µl	80 µl
Dye Reagent A	10 µl	10 µl	10 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, keep in dark for 10 minutes at room temperature, record absorbance measured at 492 nm.			

Note:

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

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

Calculation of NAD^+:

1. According to the volume of sample

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

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

2. According to the protein concentration of sample

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

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

3. According to the weight of sample

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

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

4. According to the quantity of cells or bacteria

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

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

Calculation of NADH:

1. According to the volume of sample

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

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

2. According to the protein concentration of sample

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

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

3. According to the weight of sample

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

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

4. According to the quantity of cells or bacteria

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

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

$C_{Protein}$: 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;

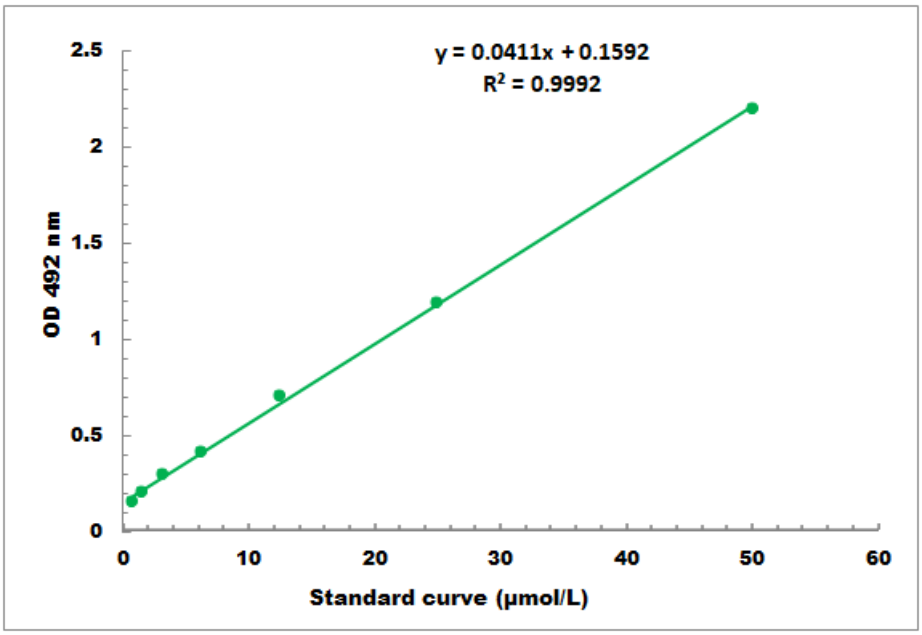
$V_{Standard}$: the volume of sample, 0.02 ml;

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

N: the quantity of cell or bacteria, $N \times 10^4$.

VII. TYPICAL DATA

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



Detection Range: 0.5 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