



NAD/NADH Microplate Assay Kit

Catalog # AS0008

Detection and Quantification of NAD/NADH Content in Urine,
Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and
Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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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 570nm, 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
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.

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

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, interval 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, interval 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 microcentrifuge tubes:

Reagent	Sample	Standard	Blank
Sample	20 μ l	--	--
Standard	--	20 μ l	--
Distilled water	--	--	30 μ l
Substrate	80 μ l	80 μ l	80 μ l
Dye Reagent	10 μ l	10 μ l	10 μ l
Stop Solution	--	--	--
Enzyme	10 μ l	10 μ l	--
Mix, keep them in dark for 2 minutes at room temperature.			
Stop Solution	200 μ l	200 μ l	200 μ l
Mix, stay at room temperature for 1 minutes, centrifuged at 20,000g for 5 minutes, discard the supernatant after centrifugation.			
Dissolution Buffer	300 μ l	300 μ l	300 μ l
Add 200 μ l solution into the microplate, record absorbance measured at 570 nm.			

VI. CALCULATION

Calculation of NAD⁺:

1. According to the volume of sample

$$\begin{aligned} \text{NAD}^+ (\mu\text{mol/ml}) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} / (V_{\text{Sample}} \\ &\quad / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

2. According to the protein concentration of sample

$$\begin{aligned} \text{NAD}^+ (\mu\text{mol/mg}) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} / (V_{\text{Sample}} \times \\ &\quad C_{\text{Protein}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

3. According to the weight of sample

$$\begin{aligned} \text{NAD}^+ (\mu\text{mol/g}) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

4. According to the quantity of cells or bacteria

$$\begin{aligned} \text{NAD}^+ (\mu\text{mol}/10^4) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

Calculation of NADH:

1. According to the volume of serum or plasma

$$\begin{aligned} \text{NADH} (\mu\text{mol/ml}) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} / (V_{\text{Sample}} \\ &\quad / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

2. According to the protein concentration of sample

$$\begin{aligned} \text{NADH} (\mu\text{mol/mg}) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} / (V_{\text{Sample}} \times \\ &\quad C_{\text{Protein}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

3. According to the weight of sample

$$\begin{aligned} \text{NADH } (\mu\text{mol/g}) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} / (W \times \\ & \quad V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

4. According to the quantity of cells or bacteria

$$\begin{aligned} \text{NADH } (\mu\text{mol}/10^4) &= C_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} / (N \times \\ & \quad V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

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

C_{Standard} : the protein concentration, 50 $\mu\text{mol/L}$ = 0.05 $\mu\text{mol/ml}$;

W: the weight of sample, g;

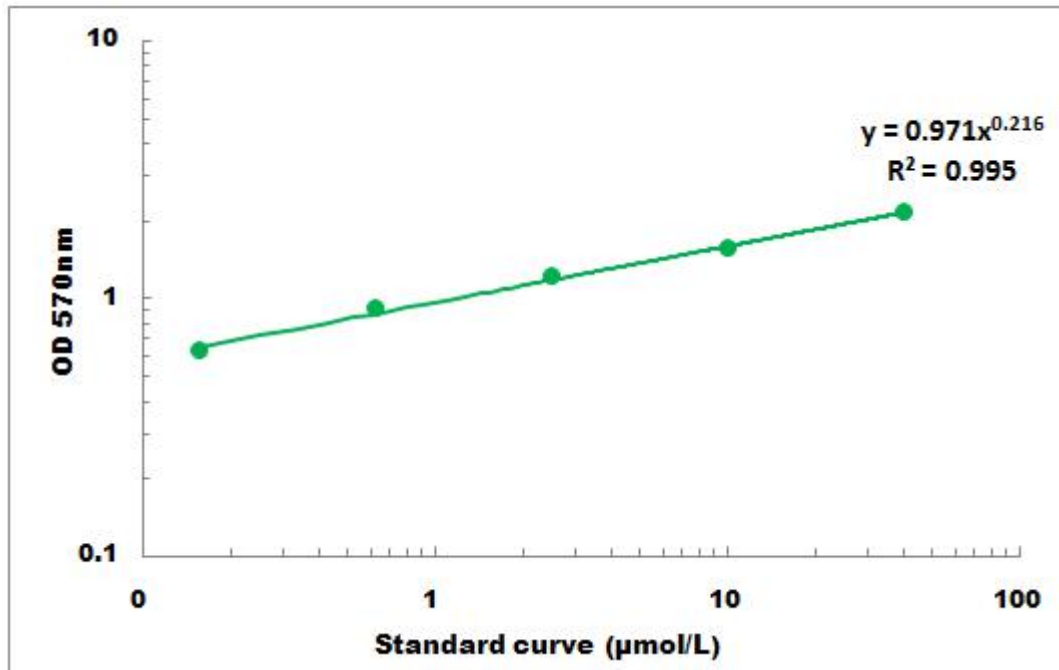
V_{Sample} : the volume of sample, 0.02 ml;

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

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

VII. TYPICAL DATA

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



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES