



NAD/NADH

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1008

(Version 2.5F)

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

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 Colorimetric 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 450nm, is proportionate to the NAD^+ /NADH concentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	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 ReagentA	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	-20 °C, keep in dark
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Water bath

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, keep in dark and store at -20 °C for 2-3 weeks after reconstitution. Then dilute to 50 µmol/L standard top solution by adding 25 µl stock solution into 975 µl distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 50/25/12.5/6.25/3.12/1.56/0.78 µmol/L.

Dye Reagent A: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, mix. Keep in dark and store at -20°C for 1 month.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use, mix. Store at -80°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For serum or plasma samples

Total NADH and NAD^+ :

Detect directly or dilute with distilled water.

NAD^+ Decomposition:

To detect NADH, the NAD^+ needs to be decomposed before the reaction. Keep some samples at 60°C for 30 min in water bath to completely decompose the NAD^+ .

Cool samples on ice. Centrifuge at $8000g$ 4°C for 10 minutes and transfer the supernatant into a new centrifuge tube, keep it on ice for detection.

2. For tissue samples

Total NADH and NAD^+ :

Weigh out 0.05g tissue, homogenize with 500 μl Assay Buffer on ice; centrifuge at $8000g$ 4°C for 10 minutes, transfer the supernatant into a new centrifuge tube, keep it on ice for detection.

NAD^+ Decomposition:

To detect NADH, the NAD^+ needs to be decomposed before the reaction. Keep some samples at 60°C for 30 min in water bath to completely decompose the NAD^+ . Cool samples on ice. Centrifuge at $8000g$ 4°C for 10 minutes and transfer the supernatant into a new centrifuge tube, keep it on ice for detection.

3. For cell and bacteria samples

Total NADH and NAD^+ :

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 500 μl Assay Buffer for 500×10^4 cell or bacteria, sonicate (with power 20%, sonication 2s, interval 1s, repeat 30 times); Centrifuge at $8000g$ 4°C for 10 minutes and transfer the supernatant into a new centrifuge tube; keep it on ice for detection.

NAD⁺ Decomposition:

To detect NADH, the NAD⁺ needs to be decomposed before the reaction. Keep some samples at 60°C for 30 min in water bath to completely decompose the NAD⁺. Cool samples on ice. Centrifuge at 8000g 4°C for 10minutes and transfer the supernatant into a new centrifuge tube, keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample** (Total)	Sample** (NADH)	Standard	Blank
Sample	20 μ l	20 μ l	--	--
Standard	--	--	20 μ l	--
Distilled water	--	--	--	20 μ l
Reaction Buffer	70 μ l	70 μ l	70 μ l	70 μ l
Enzyme	10 μ l	10 μ l	10 μ l	10 μ l
Substrate	80 μ l	80 μ l	80 μ l	80 μ l
Dye Reagent A	10 μ l	10 μ l	10 μ l	10 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l
Mix, keep in dark for 10 minutes at room temperature, record absorbance measured at 450 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C_{\text{NAD/NADH}} = \frac{(\text{OD}_{\text{Sample (total)}} - \text{OD}_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\mu\text{mol/L})$$

$$C_{\text{NADH}} = \frac{(\text{OD}_{\text{Sample(NADH)}} - \text{OD}_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\mu\text{mol/L})$$

$$C_{\text{NAD}}^+ = C_{\text{NAD/NADH}} - C_{\text{NADH}} (\mu\text{mol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the quantity of cells or bacteria

$$C_{\text{NAD/NADH}} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (total)}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol}/10^4)$$

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

$$C_{\text{NAD}}^+ = C_{\text{NAD/NADH}} - C_{\text{NADH}} (\mu\text{mol}/10^4)$$

2.2 According to the weight of sample

$$C_{\text{NAD/NADH}} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (total)}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol/g})$$

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

$$C_{\text{NAD}}^+ = C_{\text{NAD/NADH}} - C_{\text{NADH}} (\mu\text{mol/g})$$

2.3 According to the volume of sample

$$C_{\text{NAD/NADH}} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (total)}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

$$C_{\text{NADH}} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample(NADH)}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

$$C_{\text{NAD}^+} = C_{\text{NAD/NADH}^-} - C_{\text{NADH}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, $\mu\text{mol/L}$

V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

V_{Assay} : the volume of Assay Buffer, μl

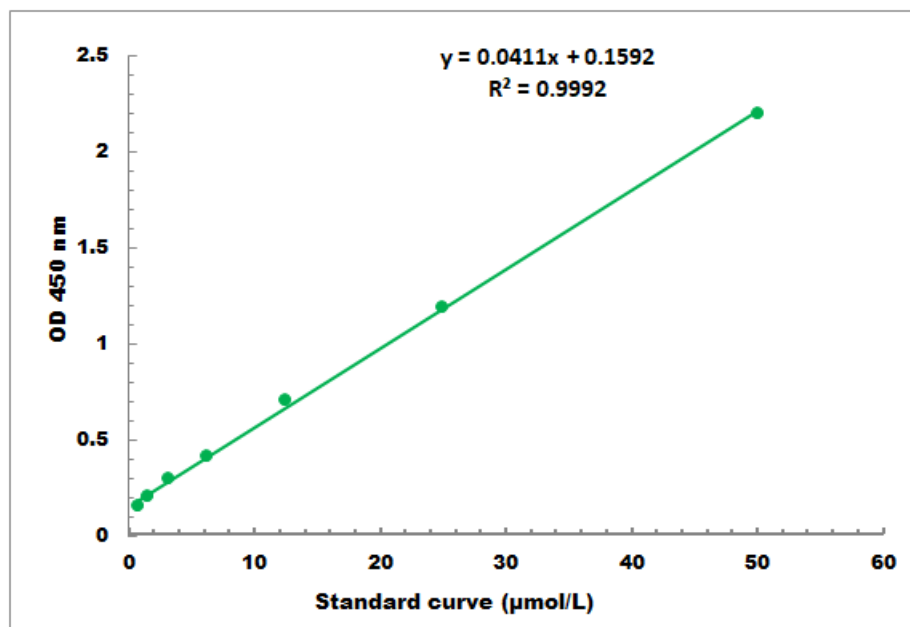
C_{Protein} : the sample protein concentration, mg/ml

W: the weight of sample, g

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

VIII. TYPICAL DATA

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



Detection Range: 0.5 $\mu\text{mol/L}$ - 50 $\mu\text{mol/L}$