NADP/NADPH

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1009

(Version 2.6F)

Detection and Quantification of NADP/NADPH 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

NADP (Nicotinamide adenine dinucleotide phosphate) is a coenzyme composed of ribosylnicotinamide 5-phosphate (NMN) coupled by pyrophosphate linkage to the 5-phosphate adenosine 2,5-biphosphate. It serves as an electron carrier in a number of reactions, being alternately oxidised (NADP+) and reduced (NADPH). The oxidative phase of the pentose phosphate pathway is the major source of NADPH in cells, producing approxiamtely 60% of the NADPH required. NADPH provides the reducing equivalents for biosynthetic reactions and the oxidation-reduction involved in protecting against the toxicity of ROS, allowing the regeneration of GSH. NADPH is also used for anabolic pathways, such as lipid synthesis, cholesterol synthesis and fatty acid chain elongation.

NADP/NADPHColorimetricMicroplate Assay Kitprovides a simple and direct procedure for measuring $NADP^+$/NADPH levels in a variety of samples. The kit is based on analcohol dehydrogenase cycling reaction, in which the formed NADPH reduces a formazan reagent. The intensity of the reduced product color, measured at 450nm, is proportionate to the $NADP^+$/NADPH 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 mlx 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.Dissolvein1 mldistilled waterto generate 2 mmol/L of standardstock solution,keep in dark and store at -20 °C for 2-3 weeksafter reconstitution.Then dilute to50 µmol/L standard top solutionby adding 25 µl stock solution into 975µl distilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be50/25/12.5/6.25/3.12/1.56/0.78µmol/L.

Dye Reagent A: Briefly centrifuge prior to opening. Add 1 mldistilled 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 mlReaction 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

TotalNADPHand $NADP^+$:

Detect directly or dilute with distilled water.

$NADP^+$ Decomposition:

To detect NADPH, the $NADP^+$ needs to be decomposed before the reaction. Keep somesamples at 60°C for 30 min in water bath to completely decompose the $NADP^+$. 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.

2.For tissue samples

TotalNADPHand $NADP^+$:

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

$NADP^+$ Decomposition:

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

3.For cell and bacteria samples

TotalNADPHand $NADP^+$:

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

$NADP^+$ Decomposition:

To detect NADPH, the $NADP^+$ needs to be decomposed before the reaction. Keep some samples at 60°C for 30 min in water bath to completely decompose the $NADP^+$. 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 intothe microplate:

Reagent*	Sample** (Total)	Sample** (NADPH)	Standard	Blank
Sample	20 µl	20 µl	--	--
Standard	--	--	20 µl	--
Distilled water	--	--	--	20 µl
Reaction Buffer	70 µl	70 µl	70 µl	70 µl
Enzyme	10 µl	10 µl	10 µl	10 µl
Substrate	80 µl	80 µl	80 µl	80 µl
Dye Reagent A	10 µl	10 µl	10 µl	10 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl
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. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C_{NADP/NADPH}=\frac{(OD_{Sample\ (total)}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(\mu mol/L)$$

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

$$C_{NADP^+}=C_{NADP/NADPH}-C_{NADPH}(\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure.

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

2.1According to the quantity of cells or bacteria

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

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

$$C_{NADP^+}=C_{NADP/NADPH}-C_{NADPH}(\mu mol/10^4)$$

2.2 According to the weight of sample

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

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

$$C_{NADP^+}=C_{NADP/NADPH}-C_{NADPH}(\mu mol/g)$$

2.3According to the volume of sample

$$C_{NADP/NADPH}=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample\ (total)}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

$$C_{NADPH}=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample(NADPH)}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

$C_{NADP}^{+}=C_{NADP/NADPH}- C_{NADPH}$(μmol/ml)

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, μmol/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

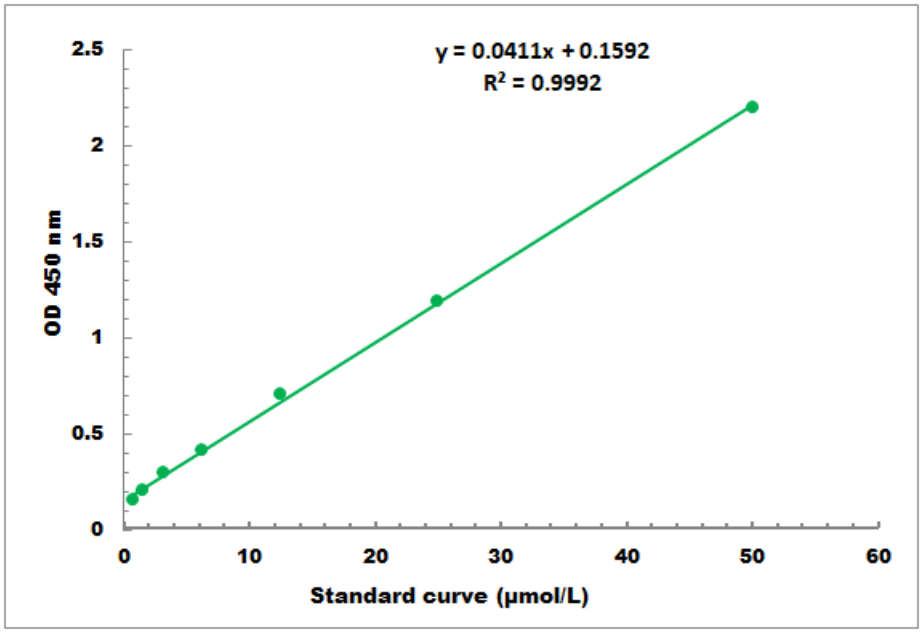
$C_{Protein}$: the sample protein concentration, mg/ml

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: 0.5μmol/L -50 μmol/L