NADP/NADPH Microplate Assay Kit

User Manual

Catalog # CAK1009

(Version 2.5F)

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/NADPH Microplate Assay Kit provides a simple and direct procedure for measuring $NADP^+$/NADPH levels in a variety of samples. The kit is based on an alcohol dehydrogenase cycling reaction, in which the formed NADPH reduces a formazan reagent. The intensity of the reduced product color, measured at 450 nm, 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 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
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 -20°C for a month.

Enzyme: add 1 ml Reaction Buffer to dissolve before use, mix. Store at -80°C for a month.

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. Store at -20°C for a month.

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. SAMPLE PREPARATION

1. For serum or plasma samples

Total NADPH and $NADP^+$:

Detect directly or dilute with distilled water.

$NADP^+$ 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 10 minutes and transfer the supernatant into a new centrifuge tube, keep it on ice for detection.

2. For tissue samples

Total NADPH and $NADP^+$:

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

3. For cell and bacteria samples

Total NADPH and $NADP^+$:

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, intervation 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.

$NADP^+$ 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 10 minutes and transfer the supernatant into a new centrifuge tube, keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents into the 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:

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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

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

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

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

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

$$NADP^+\ (\mu mol/ml) = NADP/NADPH\ (\mu mol/ml) - NADPH\ (\mu mol/ml)$$

2. According to the weight of sample

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

$$= 0.025 \times (OD_{Sample(Total)} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

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

$$= 0.025 \times (OD_{Sample(NADPH)} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

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

3. According to the quantity of cells or bacteria

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

$$= 0.025 \times (OD_{Sample(Total)} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

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

$$= 0.025 \times (OD_{Sample(NADPH)} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

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

$C_{Protein}$: 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;

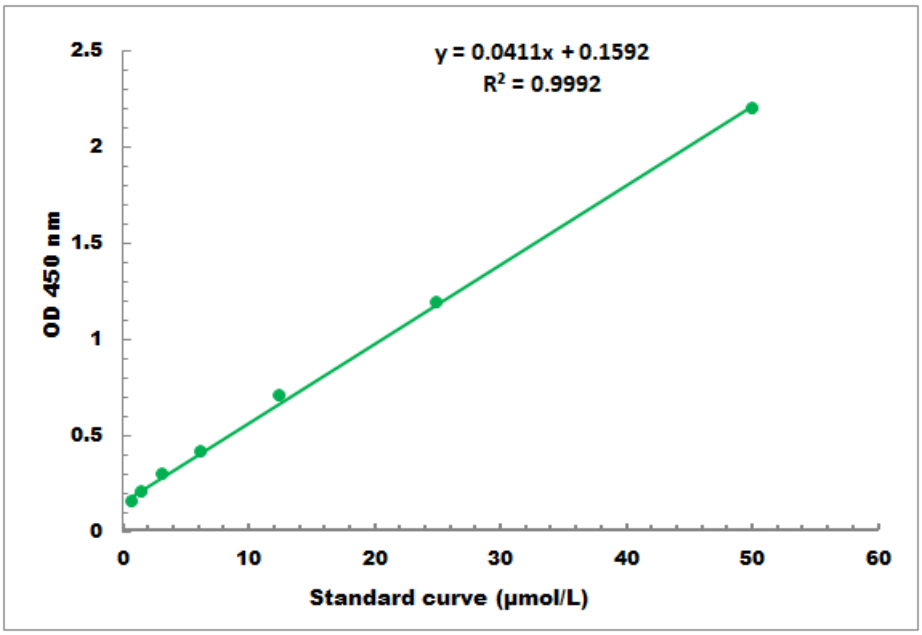
$V_{Standard}$: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay Buffer, 0.5 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