NADP/NADPH Microplate Assay Kit

User Manual

Catalog # ASK1009

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.

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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 492 nm, is proportionate to the NADP+/NADPH concentration in the sample.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 1	4 °C
Assay Buffer II	30 ml x 1	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
NADPH Standard	Powder x 1	-20 °C, keep in dark
$NADP^+$ 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.

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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

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IV. SAMPLE PREPARATION

1. For serum or plasma samples

Extract the $NADP^+$:

Add 100 μ l Assay Buffer I to 50 μ l serum or plasma; mix; incubate at 60 °C for 20 minutes; centrifuged at 4000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 100 μ l Assay Buffer II, mix; keep it on ice for detection.

Extract the NADPH:

Add 100 μ l Assay Buffer I to 50 μ l serum or plasma; mix; incubate at 60 °C for 20 minutes; centrifuged at 4000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 100 μ l Assay Buffer I, mix; keep it on ice for detection.

2. For tissue samples

Weigh out 0.05g tissue, homogenize with 100 μ l Assay Buffer I on ice; incubate at 60 °C for 20 minutes; centrifuged at 4000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 100 μ l Assay Buffer II, mix; keep it on ice for detection.

Extract the NADPH:

Weigh out 0.05g tissue, homogenize with 100 μ l Assay Buffer II on ice; incubate at 60 °C for 20 minutes; centrifuged at 4000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 100 μ l Assay Buffer I, mix; keep it on ice for detection.

3. For cell and bacteria samples

Extract the $NADP^+$:

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 100 μ l Assay Buffer I for 100 × 104 cell or bacteria, sonicate (with power 20%, sonication 2s, intervation 1s, repeat 30 times); incubate at 60 °C for 20

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minutes; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 100 μ l Assay Buffer II, mix; keep it on ice for detection.

Extract the NADPH:

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 100 μ l Assay Buffer II for 100 × 104 cell or bacteria, sonicate (with power 20%, sonication 2s, intervation 1s, repeat 30 times); incubate at 60 °C for 20 minutes; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and add 100 μ l Assay Buffer I, mix; keep it on ice for detection.

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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.

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VI. CALCULATION

Calculation of NADP⁺:

1. According to the volume of sample

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

$= 0.25 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

2. According to the weight of sample

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

$= 0.01 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the quantity of cells or bacteria

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

$= 0.01 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

Calculation of NADPH:

1. According to the volume of serum or plasma

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

$= 0.25 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

2. According to the weight of sample

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

$= 0.01 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the quantity of cells or bacteria

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

$= 0.01 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

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$C_{Protein}$: 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 I and Assay Buffer II, 0.2 ml;

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

n: dilution factor = 5.

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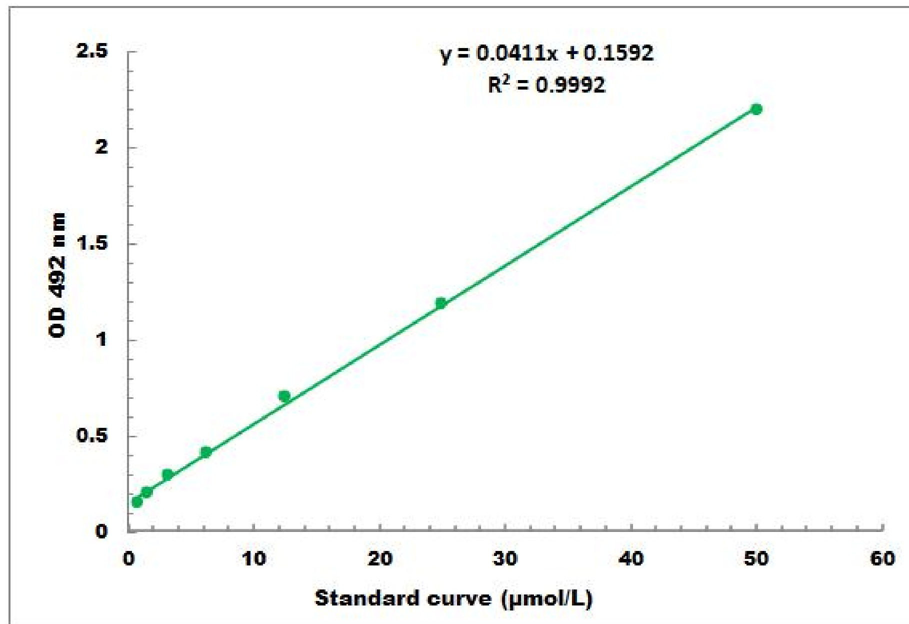
VII. TYPICAL DATA

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

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