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 is based on a glucose dehydrogenase cycling reaction, in which the formed NADPH reduces a formazan (MTT) reagent. The intensity of the reduced product color, measured at 570 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 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
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
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 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 NADPH:

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 $NADP^+$:

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 NADPH:

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 $NADP^+$:

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, intervation 1s, repeat 30 times); boiling for 5 minutes;

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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 NADPH:

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, intervation 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.

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V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

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

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VI. CALCULATION

Calculation of $NADP^+$:

1. According to the volume of sample

$NADP^+$ (μmol/ml) = $C_{Standard}$ × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) × $V_{Standard}$ / (V_{Sample} / V_{Assay})

= 0.05 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})

2. According to the protein concentration of sample

$NADP^+$ (μmol/mg) = $C_{Standard}$ × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) × $V_{Standard}$ / (V_{Sample} × $C_{Protein}$)

= 0.05 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / $C_{Protein}$

3. According to the weight of sample

$NADP^+$ (μmol/g) = $C_{Standard}$ × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) × $V_{Standard}$ / (W × V_{Sample} / V_{Assay})

= 0.05 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / W

4. According to the quantity of cells or bacteria

$NADP^+$ (μmol/10^4) = $C_{Standard}$ × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) × $V_{Standard}$ / (N × V_{Sample} /V_{Assay})

= 0.05 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / N

Calculation of NADPH:

1. According to the volume of serum or plasma

NADPH (μmol/ml) = $C_{Standard}$ × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) × $V_{Standard}$ / (V_{Sample} / V_{Assay})

= 0.05 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})

2. According to the protein concentration of sample

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

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

3. According to the weight of sample

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

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

4. According to the quantity of cells or bacteria

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

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

$C_{Protein}$: 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, 1 ml;

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

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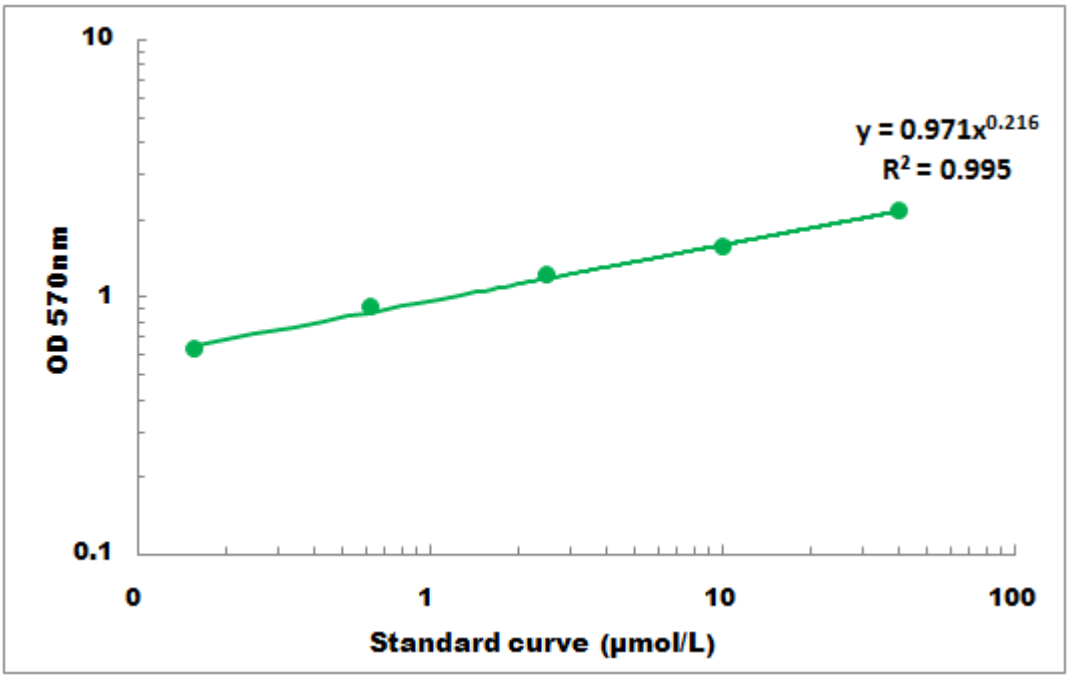
VII. TYPICAL DATA

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



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

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