



# **NADP/NADPH Microplate Assay Kit**

## **User Manual**

**Catalog # FTA0009**

(Version 2.1D)

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<sup>+</sup>) and reduced (NADPH). The oxidative phase of the pentose phosphate pathway is the major source of NADPH in cells, producing approximately 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<sup>+</sup>/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<sup>+</sup>/NADPH concentration in the sample.

## 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                 |
| 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 <sup>+</sup> 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.

**NADPH 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.

**NADP<sup>+</sup> 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.

### III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 492 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

### IV. SAMPLE PREPARATION

1. For serum or plasma samples

Extract the NADP<sup>+</sup>:

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<sup>+</sup>:

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<sup>+</sup>:

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5 ml Assay buffer I for  $250 \times 10^4$  cell or bacteria, sonicate (with power 20%, sonication 2s, interval 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 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  $250 \times 10^4$  cell or bacteria, sonicate (with power 20%, sonication 2s, interval 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.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

| Reagent   | Sample     | Standard   | Blank      |
|---|------------|------------|------------|
| Sample  | 20 $\mu$ l | --         | --         |
| Standard  | --         | 20 $\mu$ l | --         |
| Distilled water   | --         | --         | 20 $\mu$ l |
| Reaction Buffer   | 70 $\mu$ l | 70 $\mu$ l | 70 $\mu$ l |
| Enzyme  | 10 $\mu$ l | 10 $\mu$ l | 10 $\mu$ l |
| Substrate   | 80 $\mu$ l | 80 $\mu$ l | 80 $\mu$ l |
| Dye Reagent A   | 10 $\mu$ l | 10 $\mu$ l | 10 $\mu$ l |
| Dye Reagent B   | 10 $\mu$ l | 10 $\mu$ l | 10 $\mu$ l |
| 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.

## VI. CALCULATION

### Calculation of NADP<sup>+</sup>:

1. According to the volume of sample

$$\begin{aligned} \text{NADP}^+ (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

2. According to the protein concentration of sample

$$\begin{aligned} \text{NADP}^+ (\mu\text{mol/mg}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

3. According to the weight of sample

$$\begin{aligned} \text{NADP}^+ (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \\ &\quad \times V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

4. According to the quantity of cells or bacteria

$$\begin{aligned} \text{NADP}^+ (\mu\text{mol}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (N \times V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

### Calculation of NADPH:

1. According to the volume of serum or plasma

$$\begin{aligned} \text{NADPH} (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

2. According to the protein concentration of sample

$$\begin{aligned} \text{NADPH} (\mu\text{mol/mg}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$



3. According to the weight of sample

$$\begin{aligned}\text{NADPH } (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \\ &\quad \times V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

4. According to the quantity of cells or bacteria

$$\begin{aligned}\text{NADPH } (\mu\text{mol}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (N \times V_{\text{Sample}}/V_{\text{Assay}}) \\ &= 0.05 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

$C_{\text{Standard}}$ : the protein concentration, 50  $\mu\text{mol/L}$  = 0.05  $\mu\text{mol/ml}$ ;

$W$ : the weight of sample, g;

$V_{\text{Sample}}$ : the volume of sample, 0.02 ml;

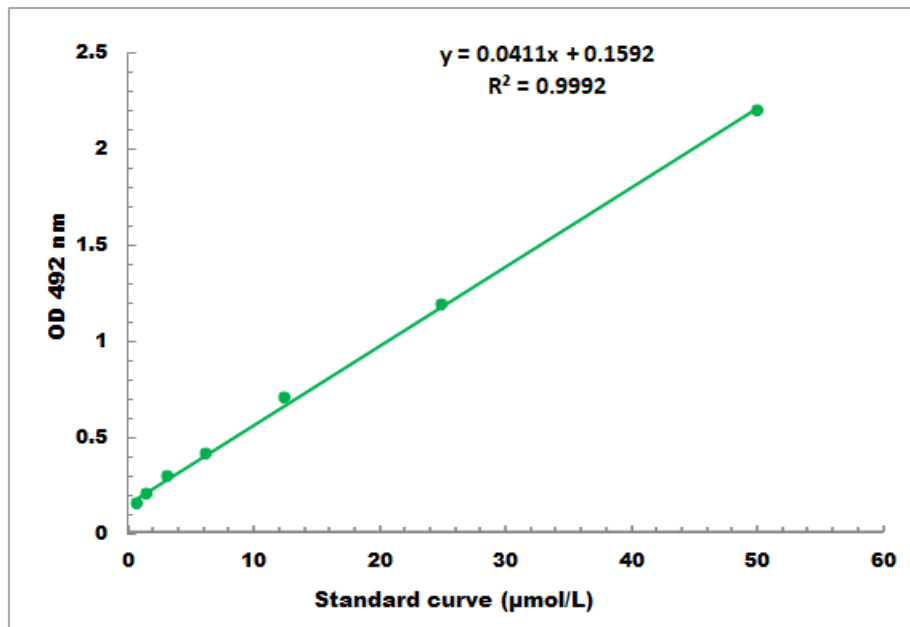
$V_{\text{Standard}}$ : the volume of sample, 0.02 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 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](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES