



NADH Oxidase Microplate Assay Kit

User Manual

Catalog # CAK1044

(Version 1.5G)

Detection and Quantification of NADH Oxidase (NOX) Activity in
Tissue extracts, Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

NADH oxidase is a cyanide-resistant and hormone-responsive oxidase intrinsic to the plasma membrane of both plant and animal cells. The activity has many unique characteristics that distinguish it from other oxidases and oxidoreductases of both organelles and internal membranes and from other oxidoreductases of the plasma membrane. Among these are resistance to inhibition by cyanide, catalase, superoxide dismutase, and phenylchloromercuribenzoate. Activity is stimulated by hormones and growth factors and inhibited by quinone analogs such as piercidin, the flavin antagonist atebirin, and growth inhibiting gangliosides such as GM3.

NADH Oxidase Microplate Assay Kit provides a simple and direct procedure for measuring NADH Oxidase activity levels. The assay is initiated with the enzymatic hydrolysis of the NADH by NOX. The enzyme catalysed reaction products can be measured at a colorimetric readout at 450 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	1.2 ml x 1	4 °C
Assay Buffer III	20 ml x 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 1 ml distilled water to dissolve before use.

Dye Reagent A: add 9 ml distilled water to dissolve before use, mix, store at 4°C.

Standard: add 1 ml distilled water to dissolve before use; then add 0.3 ml into 0.7 ml distilled water, the concentration will be 600 µmol/L.

Positive Control: add 1 ml Assay Buffer to dissolve before use, mix.

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. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.99 ml Assay Buffer I and 10 μ l Assay Buffer II on ice, centrifuged at 600g 4 °C for 5 minutes. Take the supernatant into a new centrifuge tube, 10000g 4 °C for 10 minutes, discard the supernatant. Add 198 μ l Assay Buffer III and 2 μ l Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 0.99 ml Assay Buffer I and 10 μ l Assay Buffer II on ice, centrifuged at 600g 4 °C for 5 minutes. Take the supernatant into a new centrifuge tube, 10000g 4 °C for 10 minutes, discard the supernatant. Add 198 μ l Assay Buffer III and 2 μ l Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times). Centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	80 µl	80 µl	--	--	80 µl
Substrate	10 µl	10 µl	--	--	10 µl
Sample	10 µl	--	--	--	--
Positive Control	--	--	--	--	10 µl
Standard	--	--	100 µl	--	--
Distilled water	--	10 µl	--	100 µl	--
Dye Reagent A	90 µl	90 µl	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, cover the plate adhesive strip, put the plate into the convection oven, incubate at 37 °C for 5 minutes, measured at 450 nm and record the absorbance.					

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: one unit of NOX activity is defined as the enzyme oxidize 1 μmol NADH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{NOX (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 1.2 \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{NOX (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad W / V_{\text{Assay}}) / T \\ &= 1.2 \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{NOX (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) (V_{\text{Sample}} \\ &\quad \times N / V_{\text{Assay}}) / T \\ &= 1.2 \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of sample

$$\begin{aligned} \text{NOX (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &\quad / T \\ &= 1.2 \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

C_{Standard} : the standard concentration, 600 $\mu\text{mol/L}$ = 0.6 $\mu\text{mol/ml}$;

V_{Standard} : the volume of standard, 100 μl = 0.1 ml;

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

W: the weight of sample, g;

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

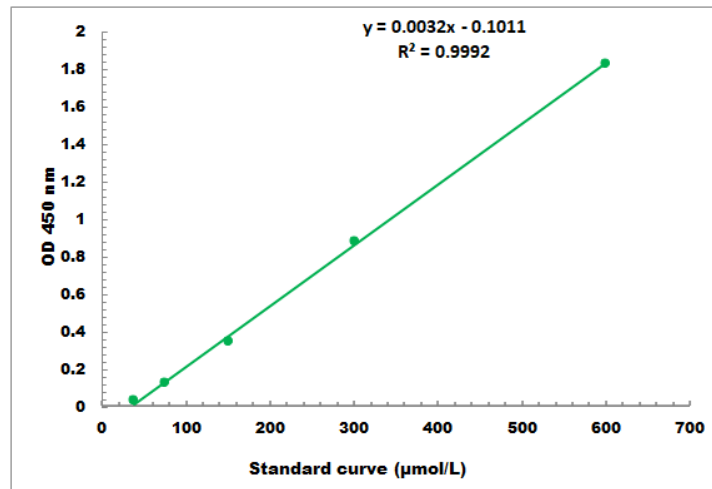
V_{Sample} : the volume of sample, 0.01 ml;

V_{Assay} : the volume of Assay buffer, 1 ml;

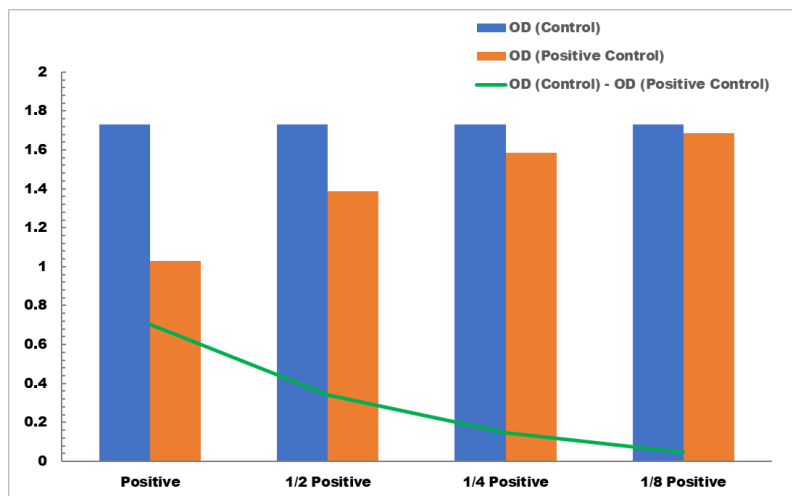
T: the reaction time, 5 minutes.

VII. TYPICAL DATA

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



Detection Range: 30 µmol/L - 600 µmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES