



NADH Oxidase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1044

(Version 1.6G)

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 piericidin, the flavin antagonist atebirin, and growth inhibiting gangliosides such as GM3.

NADH Oxidase Activity Colorimetric 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 mlx 4	4 °C
Assay Buffer II	1.2 mlx 1	4 °C
Assay Buffer III	20 mlx 1	4 °C
Reaction Buffer	10 mlx 1	4 °C
Substrate	Powder x 1	-20 °C
Dye ReagentA	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	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution. Keep in dark for immediate use or store at -20 °C for 2-4 weeks. Then dilute to 400 $\mu\text{mol/L}$ standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 $\mu\text{mol/L}$.

Substrate: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use. Keep in dark for immediate use or store at -20 °C for 2-4 weeks.

Dye Reagent A: Add 9 ml distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use, mix. Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. 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, measure at 450 nm and record the absorbance.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

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

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10⁴

C_{Standard}: the concentration of standard, μ mol/L

V_{Standard}: the volume of standard in assay procedure, mL

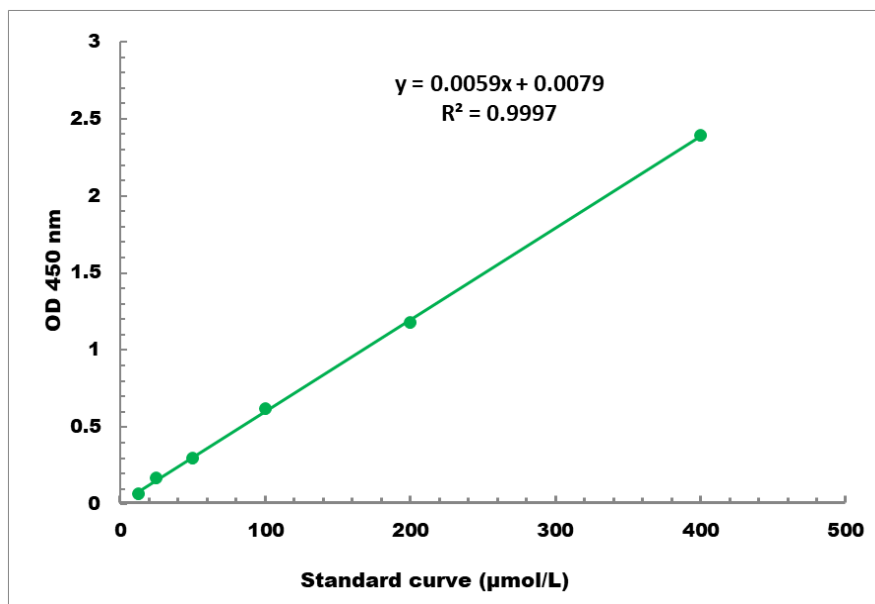
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer II and Assay Buffer III in sample preparation, mL

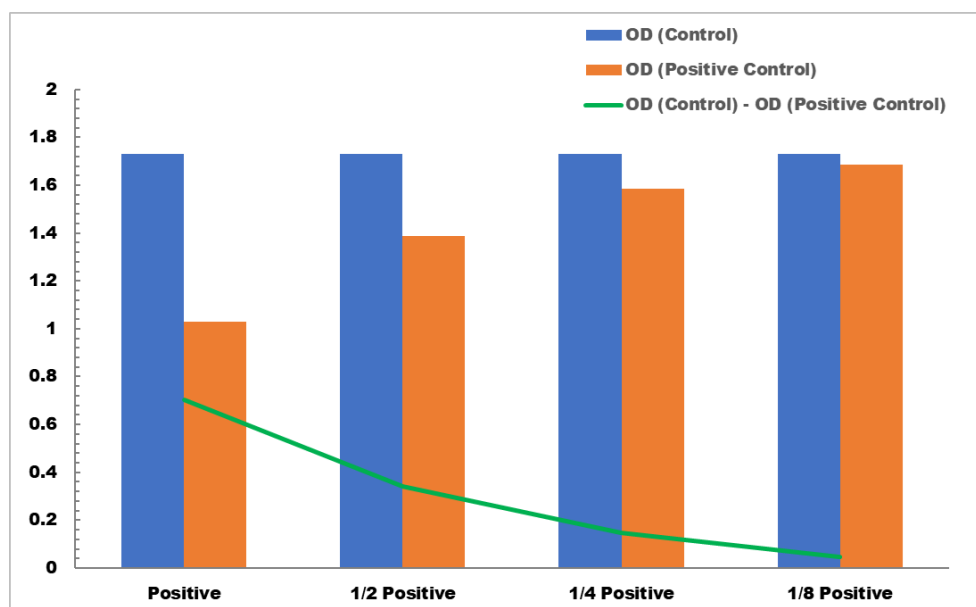
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 4μmol/L -400 μmol/L



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