NADH Oxidase Microplate Assay Kit

User Manual

Catalog # ASK1044

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 an enzyme that uses NADH and oxygen to produce H2O2 and NAD+. 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 600 nm.

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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
Substrate	Powder x 1	-20 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	15 ml x 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate: add 5 ml distilled water to dissolve before use, store at -20 °C.

Dye Reagent: add 15 ml Dye Reagent Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 600 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

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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 µl 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 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonication 3s, intervation 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 µl 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 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonication 3s, intervation 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.

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V. ASSAY PROCEDURE

Warm the Dye Reagent to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample
Substrate	40 µl
Dye Reagent	150 µl
Mix.	
Sample	10 µl
Mix, measured at 600 nm and record the absorbance of 10th second and 70th second.	

Note: if the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time.

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VI. CALCULATION

Unit Definition: one unit is defined as the OD_{600} value changed 0.01 in the per ml reaction system per minute.

1. According to the protein concentration of sample

$$NOX\ (U/mg) = (OD_{Sample(10S)} - OD_{Sample(70S)}) \times V_{Total} / (V_{Sample} \times C_{Protein}) / T / 0.01$$

$$= 2000 \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / C_{Protein}$$

2. According to the weight of sample

$$NOX\ (U/g) = (OD_{Sample(10S)} - OD_{Sample(70S)}) \times V_{Total} / (V_{Sample} \times W / V_{Assay}) / T / 0.01$$

$$= 400 \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / W$$

3. According to the quantity of cells or bacteria

$$NOX\ (U/10^4) = (OD_{Sample(10S)} - OD_{Sample(70S)}) \times V_{Total} / (V_{Sample} \times N / V_{Assay}) / T / 0.01$$

$$= 400 \times (OD_{Sample(10S)} - OD_{Sample(70S)}) / N$$

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

W: the weight of sample, g;

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

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

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

V_{Assay}: the volume of Assay buffer, 0.2 ml;

T: the reaction time, 1 minute.

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