

**NADH Dehydrogenase
(Mitochondrial Complex I)
Microplate Assay Kit
Cat #: orb707330 (manual)**

Detection and Quantification of NADH Dehydrogenase (Mitochondrial Complex I) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

INTRODUCTION

NADH dehydrogenase (EC 1.6.5.3) (also referred to as NADH: ubiquinone oxidoreductase or, especially in the context of the human protein, Complex I) is an enzyme of the respiratory chains of myriad organisms from bacteria to humans that falls under the H⁺ or Na⁺-translocating NADH Dehydrogenase (NDH) Family, a member of the Na⁺ transporting Mrp superfamily. It catalyzes the transfer of electrons from NADH to coenzyme Q10 (CoQ10) and, in eukaryotes, it is located in the inner mitochondrial membrane. NADH: ubiquinone oxidoreductases type I of bacteria and of eukaryotic mitochondria and chloroplasts couple electron transfer to the electrogenic transport of protons or Na⁺. It is one of the "entry enzymes" of cellular respiration or oxidative phosphorylation in the mitochondria.

The assay is used to determine NADH Dehydrogenase. The enzyme catalysated reaction products is NAD⁺. The reaction velocity is measured as a decrease in A340 resulting from the oxidation of NADH.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

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	20 ml x 1	-20 °C
Inhibitor	Powder x 1	-20 °C
Inhibitor Diluent	0.5 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Substrate	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
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Note:

Inhibitor: add 0.5 ml Inhibitor Diluent to dissolve before use.

Enzyme: add 1 ml Reaction Buffer to dissolve before use.

Substrate: add 1 ml Reaction Buffer to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

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, 11000g 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 11000g 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, 11000g 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 11000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 μ l	--
Distilled water	--	--	200 μ l
Reaction Buffer	165 μ l	--	--
Inhibitor	5 μ l	--	--
Enzyme	10 μ l	--	--
Substrate	10 μ l	--	--
Sample	10 μ l	--	--

Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.

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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION

Unit Definition: One unit of Complex I activity is defined as the enzyme oxidizes 1 nmol of NADH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{Complex I (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad C_{\text{Protein}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{Complex I (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times W \\ &\quad / V_{\text{Assay}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{Complex I (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad N / V_{\text{Assay}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of serum or plasma

$$\begin{aligned} \text{Complex I (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 4000 \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

C_{Standard} : the standard concentration, 400 $\mu\text{mol/L} = 400 \text{ nmol/ml}$;

V_{Standard} : the volume of standard, 200 $\mu\text{l} = 0.2 \text{ ml}$;

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

W: the weight of sample, g;

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

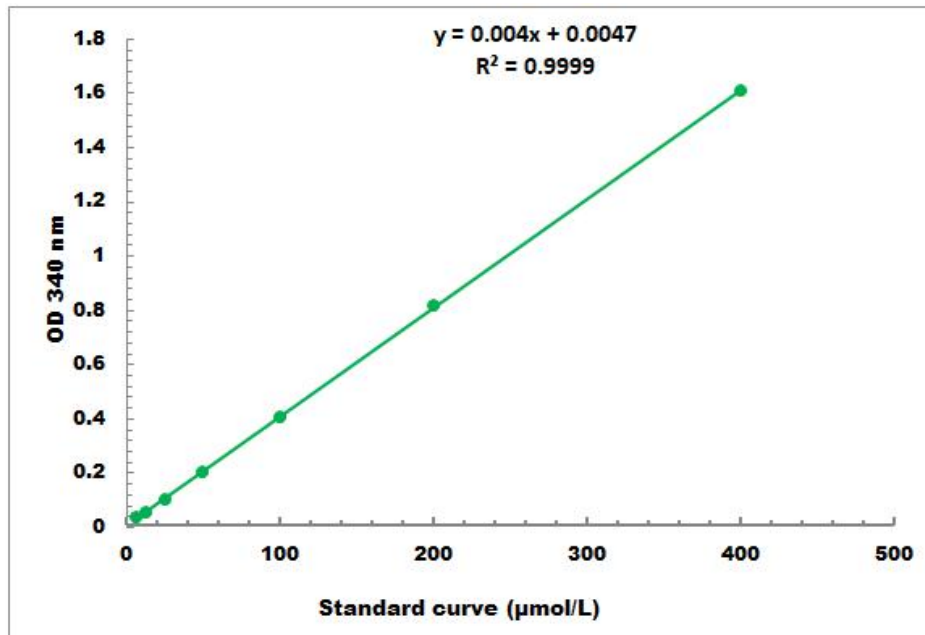
V_{Sample} : the volume of sample, 0.01 ml;

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

T: the reaction time, 2 minutes.

TYPICAL DATA

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



Detection Range: 4 µmol/L - 400 µmol/L