NADH Dehydrogenase (Mitochondrial Complex I) Activity Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1123

(Version 1.7B)

Detection and Quantification ofNADH Dehydrogenase(MitochondrialComplex I) ActivityinTissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

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I. 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.

NADH Dehydrogenase ActivityColorimetric Microplate Assay Kit provides a sensitive and convenient method for NADH dehydrogenase activity determination. The enzyme catalysed reaction productsisNAD^+.The reaction velocity is measured as a decrease in A450 resulting from the oxidation of NADH.

III.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	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	20 mlx 1	-20 °C
Inhibitor	Powder x 1	-20 °C
Inhibitor Diluent	0.5 mlx 1	4 °C
Enzyme	Powder x 1	-20 °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
Technical Manual	1 Manual	

II. 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
8. Ice

IV. REAGENTPREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 2 mmol/L of standardstock solution, store at 4 °C for 1 day or -20°C for 3daysafter reconstitution.Then dilute to400µmol/Lstandard top solutionby adding0.2 ml stock solution into 0.8 mldistilled water.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be400/200/100/50/25/12.5/6.25µmol/L.

Inhibitor:Briefly centrifuge prior to opening. Add 0.5 ml Inhibitor Diluentto dissolve before use.Keep in dark for immediate use or store at -20°C for 2-3months after reconstitution.

Enzyme: Add 10 ml Reaction Bufferto dissolve before use, heated at 40-50 °C.Prepare for immediate use or store at -80°C for 2 weeksafter reconstitution.

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

Dye Reagent A: Briefly centrifuge prior to opening. Add 3 ml distilled water to dissolve before use, mix, store at 4°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 0.99mlAssay Buffer Iand 10 µl 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 198µlAssay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times). Centrifuged at 11000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 0.99mlAssay Buffer Iand 10 µl Assay Buffer IIon 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 198µlAssay Buffer III and 2 µl Assay Buffer II to the precipitation, shock,sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times). Centrifuged at 11000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Standard	Blank
Reaction Buffer	35µl	35µl	--	--
Inhibitor	5µl	5µl	--	--
Enzyme	100 µl	100 µl	--	--
Substrate	10 µl	10 µl	--	--
Distilled water	--	10 µl	60 µl	160 µl
Sample	10 µl	--	--	--
Mix andincubate at room temperature for 5 minutes.				
Standard	--	--	100 µl	--
Dye Reagent A	30 µl	30 µl	30 µl	30 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl
Mix andincubate at room temperature for 2 minutes, record absorbance measured at 450 nm.				

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: Oneunit ofComplex Iactivity is defined asthe enzyme oxidizes1μmol of NADH per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

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, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

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

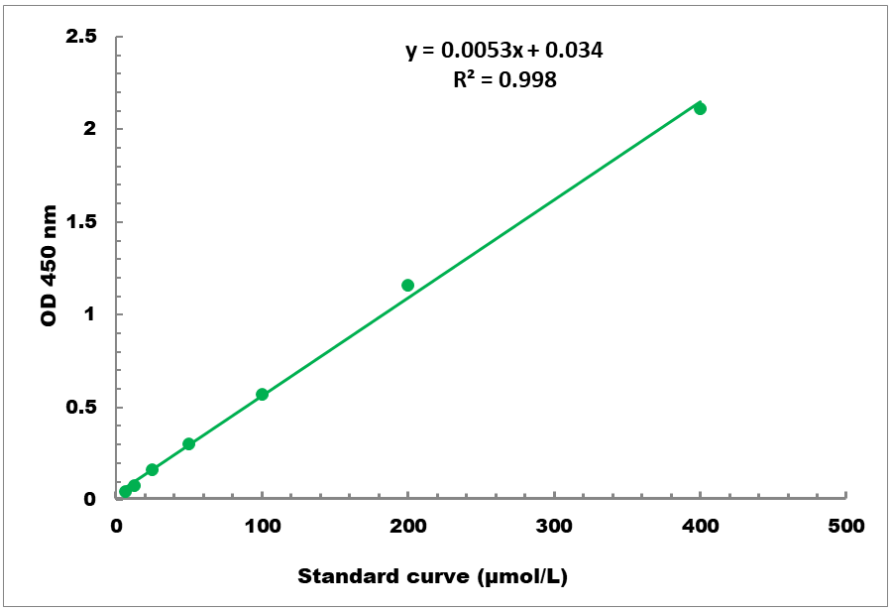
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer 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