ab284530 – NADH Oxidase Activity Assay Kit (Colorimetric)

For the quantitative measurement of NADH Oxidase.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284530

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer XII/NADH Oxidase Assay Buffer	25 mL	-20°C
NADH Oxidase Enzyme mix	1 vial	-20°C
NADH Oxidase Positive Control	1 vial	-20°C
Electron Probe/NADH Oxidase Probe	0.2 mL	-20°C
DCIP Standard/NADH Oxidase Standard	0.4 mL	-20°C
NADH Oxidase Substrate I	50 µL	-20°C
NADH Oxidase Substrate II	200 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- dH_2O
- Dounce Tissue Homogenizer
- 96-well clear flat-bottom plate
- Temperature-controlled plate reader

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Assay Buffer XII/NADH Oxidase Assay Buffer: Warm to room temperature (RT) before use. Store at 4 °C.

Electron Probe/NADH Oxidase Probe: Ready to use as supplied. Warm to RT before use. Store at -20 °C.

NADH Oxidase Enzyme mix: Reconstitute the vial in 220 µl Assay Buffer XII/NADH Oxidase Assay Buffer. Store at -20 °C. Keep on ice while in use.

NADH Oxidase Substrate I: Add 450 µl Assay Buffer XII/NADH Oxidase Assay Buffer to the vial. Store at -20 °C. Keep on ice while in use.

NADH Oxidase Substrate II: Add 1.8 ml Assay Buffer XII/NADH Oxidase Assay Buffer to the vial. Store at -20 °C. Keep on ice while in use.

NADH Oxidase Positive Control: Reconstitute the vial with 150 µl Assay Buffer XII/NADH Oxidase Assay Buffer. Divide into aliquots and store at -20 °C. Avoid multiple freeze-thaw of the enzyme. Use within six months after reconstitution. Keep on ice while in use.

DCIP Standard/NADH Oxidase Standard: Ready to use as supplied. Keep on ice while in use. Store at -20 °C.

Assay Protocol

Sample Preparation:

1. Homogenize tissue (10 mg) or cells (1 x 10^6) with 200 µl ice cold Assay Buffer XII/NADH Oxidase Assay Buffer on ice using Dounce tissue homogenizer.
2. Centrifuge the lysates at 10000 x g and 4 °C for 10 min to remove cell debris and save the supernatant.
3. Add 1-50 µl of Sample supernatant into a 96 well clear plate with flat bottom. Bring the volume of all Sample wells to 50 µl with Assay Buffer XII/NADH Oxidase Assay Buffer.
4. Prepare one well as Blank well and add 50 µl of Assay Buffer XII/NADH Oxidase Assay Buffer only.

Δ Note: *For Unknown Samples, we suggest testing several doses of your Sample to make sure the readings are within the Standard Curve range.*

Standard Curve Preparation:

1. Add 0, 4, 8, 12, 16 and 20 µl of the 2 mM DCIP Standard/NADH Oxidase Standard into a series of wells in 96-well clear plate to generate 0, 8, 16, 24, 32, and 40 nmol/well of DCIP Standard/NADH Oxidase Standard.
2. Adjust the volume of all Standard wells to 50 µl with Assay Buffer XII/NADH Oxidase Assay Buffer.

NADH Oxidase Positive Control:

1. Add 10-15 µl of the reconstituted NADH Oxidase Positive Control into the desired well(s).
2. Adjust the volume to 50 µl/well with Assay Buffer XII/NADH Oxidase Assay Buffer.

Reaction Mix:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

Item	Reaction Mix	Standard Mix
Assay Buffer XII/NADH Oxidase Assay Buffer	32 µL	34 µL
NADH Oxidase Enzyme mix	2 µL	2 µL
NADH Oxidase Substrate I	4 µL	4 µL
NADH Oxidase Substrate II	10 µL	10 µL
Electron Probe/NADH Oxidase Probe	2 µL	---

2. Mix well. Add 50 µl of Reaction Mix into Positive Control and Sample wells and 50 µl of Standard Mix into Standard wells respectively.

Measurement

Measure the absorbance (OD $_{600\ nm}$) in kinetic mode for 30 min at 25 °C.
Δ Note: *Incubation time depends on the NADH Oxidase activity in the Samples. We recommend measuring the absorbance in kinetic mode and choosing any two time points (T_1 and T_2) in the*

linear range to calculate the NADH Activity. The DCIP Standard/NADH Oxidase Standard Curve can be read in Endpoint mode (at the end of 30 min incubation).

Calculation:

1. Subtract 0 Standard reading from all Standard readings and plot the NADH Oxidase Standard Curve.
2. Subtract the Sample readings from the Blank readings to get the corrected Sample reading.
3. Apply the corrected Sample reading to the NADH Oxidase Standard Curve to get 'B' nmol of product generated during the reaction time ($\Delta T = T_2 - T_1$).
4. To determine the activity of NADH Oxidase in Sample(s), use the following equation:

$$\text{Sample NADH Oxidase Activity} = \frac{B}{\Delta T \times P} = \mathbf{nmol/min/mg} = \mathbf{mU/mg}$$

Where: **B** = Product amount from the DCIP Standard/NADH Oxidase Standard Curve (nmol)
ΔT = Difference between T_2 and T_1 (min)
P = Amount of protein in the Sample (mg)

Technical Support

For all technical or commercial enquiries please go to:

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www.abcam.co.jp/contactus (Japan)