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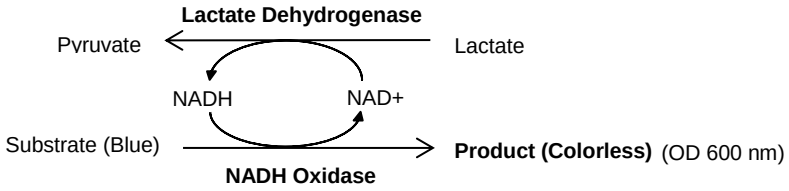
NADH Oxidase Activity Assay Kit (Colorimetric)

rev 06/21

(Catalog # K2028-100; 100 assays; Store at -20 °C)

I. Introduction:

NADH oxidase (Nicotinamide Adenine Dinucleotide Phosphate Oxidase, NOX) is a flavoprotein that catalyzes the oxidation of NADH by molecular oxygen to yield NAD^+. NOX is one of the well-known cancer biomarkers and is shown to be stimulated by growth factors and hormones. Several studies revealed the high activity of NADH oxidase in cancer patients as compared to the healthy volunteers. More importantly, inhibition of NOX correlates with the reduced cancer cell growth and induction of apoptosis. RNA interference targeting NOX expression in cancer cells effectively restored the non-cancerous phenotypes, further supporting the vital role of NOX in cancer progression. Furthermore, NOX is critical for the regeneration of NAD^+ for use in glycolysis, and for the reduction of oxygen, thereby preventing the formation of damaging reactive oxygen species. This makes NADH oxidase an attractive therapeutic target. **BioVision's NADH Oxidase Activity Assay Kit** couples the oxidation of NADH by NOX and the reduction of a colored substrate leading to a colorless product. This results in the decrease of absorbance measured at 600 nm. The kit can be used to determine NOX activity in a variety of sample types with a detection limit of ~ 1 mU of NOX activity per reaction.



II. Applications:

- Measurement of NADH oxidase activity in various tissues/cells
- Analysis of ROS in various samples
- Mechanistic studies of various cancers
- Screening anti-cancer drugs

III. Sample Types:

- Animal tissues: Liver, muscle, heart etc.
- Cell culture: adherent or suspension cells

IV. Kit Contents:

Components	K2028-100	Cap Code	Part Number
NADH Oxidase Assay Buffer	25 ml	WM	K2028-100-1
NADH Oxidase Probe	0.2 ml	Red	K2028-100-2
NADH Oxidase Enzyme mix	1 vial	Green	K2028-100-3
NADH Oxidase Substrate I	50 µl	Amber	K2028-100-4
NADH Oxidase Substrate II	200 µl	White	K2028-100-5
NADH Oxidase Positive Control	1 vial	Orange	K2028-100-6
NADH Oxidase Standard	0.4 ml	Yellow	K2028-100-7

V. User Supplied Reagents and Equipment:

- dH_2O
- Dounce Tissue Homogenizer (BioVision Cat. # 1998)
- 96-well clear plate with flat bottom
- Temperature-controlled plate reader

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store kit at -20 °C, protected from light. **Briefly centrifuge all small vials prior to opening**. Read the entire protocol before performing the assay.

- **NADH Oxidase Assay Buffer:** Warm to room temperature (RT) before use. Store at 4 °C.
- **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 NADH Oxidase Assay Buffer. Store at -20 °C. Keep on ice while in use.
- **NADH Oxidase Substrate I:** Add 450 µl 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 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 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.
- **NADH Oxidase Standard (100 mM):** Ready to use as supplied. Keep on ice while in use. Store at -20 °C.

VII. NADH Oxidase Activity Assay Protocol:

1. Sample Preparation: Homogenize tissue (10 mg) or cells (1 x 10^6) with 200 µl ice cold NADH Oxidase Assay Buffer on ice using Dounce tissue homogenizer (BioVision Cat. # 1998). Centrifuge the lysates at 10000 *x g* and 4 °C for 10 min to remove cell debris and save the supernatant. 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 NADH Oxidase Assay Buffer. Prepare one well as Blank well and add 50 µl of NADH Oxidase Assay Buffer only.

Note:

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

2. Standard Curve Preparation: Add 0, 4, 8, 12, 16 & 20 µl of the 100 mM 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 NADH Oxidase Standard. Adjust the volume of all Standard wells to 50 µl with NADH Oxidase Assay Buffer.

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

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

	Reaction Mix	Standard Mix
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
NADH Oxidase Probe	2 µl	---

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

5. Measurement: Measure the absorbance (OD $_{600\ nm}$) in kinetic mode for 30 min at 25 °C. **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 & T_2) in the linear range to calculate the NADH Activity. The NADH Oxidase Standard Curve can be read in Endpoint mode (at the end of 30 min incubation).

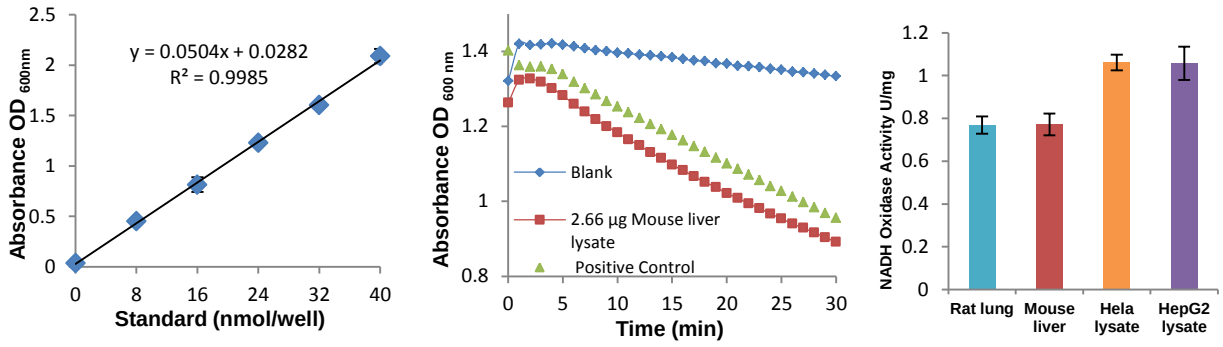
6. Calculation: Subtract 0 Standard reading from all Standard readings and plot the NADH Oxidase Standard Curve. Subtract the Sample readings from the Blank readings to get the corrected Sample reading. Apply the corrected Sample reading to the NADH Oxidase Standard Curve to get **'B'** nmol of product generated during the reaction time ($\Delta T = T_2 - T_1$).

To determine the activity of NADH Oxidase in Sample(s), use the following equation:

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

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

(A) **(B)** **(C)**



Figures: (A) NADH Oxidase Standard Curve. (B) Reaction kinetics of recombinant NADH Oxidase (Positive Control) and Mouse liver lysate. (C) NADH Oxidase activity detected in Rat lung lysate (2.8 µg), Mouse liver lysate (2.66 µg), Hela cell lysate (6 µg) and HepG2 cell lysate (3 µg). Assays were performed according to the kit protocol.

VIII. Related Products:

PicoProbe™ LDH-Cytotoxicity Fluorometric Assay Kit (# K314)
PicoProbe™ NADH Fluorometric Assay Kit (# K338)
Lactate Dehydrogenase A Inhibitor Screening Kit (# K492)
Glucose Dehydrogenase Activity Colorimetric Assay Kit (# K786)
NAD/NADH Quantitation Colorimetric Kit (# K337)
PicoProbe™ NADPH Quantitation Fluorometric Assay Kit (# K349)
Lactate Dehydrogenase Colorimetric Assay Kit (# K726)
EZScreen™ NAD+/NADH Colorimetric Assay Kit (384-well) (# K958)

FOR RESEARCH USE ONLY! Not to be used on humans.