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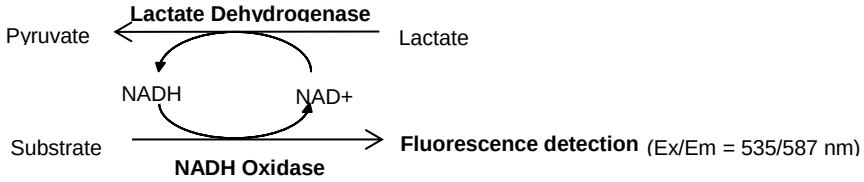
NADH Oxidase Activity Assay Kit (Fluorometric)

09/19

(Catalog # K2019-100; 100 assays; Store at -20°C)

I. Introduction:

NADH oxidase (Nicotinamide Adenine Dinucleotide Phosphate Oxidase, NOX) is a homotetrameric flavoenzyme 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 healthy volunteers. More importantly, inhibition of NOX correlates with reduced cancer cell growth and induction of apoptosis. RNA interference targeting NOX expression in cancer cells effectively restored the non-cancerous phenotypes thereby further supporting the vital role of NOX in cancer progression. Furthermore, NADH oxidase 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 oxidation of NADH by NOX and reduction of a colorless probe to a brightly colored product generating fluorescence at Ex/Em = 535/587 nm. The fluorescence generated is directly proportional to the NOX activity in samples. The kit can be used to determine NOX activity in a variety of sample type with a detection limit of ~ 15 µU of NOX activity per reaction.



II. Applications:

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

III. Sample Type:

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

IV. Kit Contents:

Components	K2019-100	Cap Code	Part Number
NADH Oxidase Assay Buffer	25 ml	WM	K2019-100-1
NADH Oxidase Lysis Buffer	25 ml	NM	K2019-100-2
PicoProbe™	0.4 ml	Blue	K2019-100-3
NADH Oxidase Enzyme mix	1 vial	Green	K2019-100-4
NADH Oxidase Substrate I	20 µl	Amber	K2019-100-5
NADH Oxidase Substrate II	300 µl	White	K2019-100-6
NADH Oxidase Developer	2 vials	Violet	K2019-100-7
NADH Oxidase Positive Control	1 vial	Orange	K2019-100-8
L (+) Lactate Standard	100 µl	Yellow	K2019-100-9

V. User Supplied Reagents and Equipment:

- dH_2O
- 96-well white opaque 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 & NADH Oxidase Lysis Buffer:** Warm to room temperature (RT) before use. Store at 4°C.
- **PicoProbe™:** Ready to use as supplied. Warm to RT before use. Store at -20°C.
- **NADH Oxidase Enzyme mix:** Reconstitute the vial with 220 µl NADH Oxidase Assay Buffer. Store at -20°C. Keep on ice while in use.
- **NADH Oxidase Substrate I:** Dilute with 200 µl NADH Oxidase Assay Buffer. Store at -20°C. Keep on ice while in use.
- **NADH Oxidase Substrate II (100 mM):** Dilute NADH Oxidase Substrate II to 10 mM by adding 300 µl of 100 mM NADH Oxidase Substrate II to 2.7 ml NADH Oxidase Assay Buffer. Mix well. Aliquot and store at -20°C. Keep on ice while in use.
- **NADH Oxidase Developer:** Reconstitute each vial with 210 µl NADH Oxidase Assay Buffer. Store at -20°C. Keep on ice while in use.
- **NADH Oxidase Positive Control:** Reconstitute the vial with 200 µl NADH Oxidase Assay Buffer. Aliquot and store at -20°C. Avoid multiple freeze/thaw of the enzyme. Use within six months after reconstitution. Keep on ice while in use.
- **L (+) Lactate 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 Lysis Buffer on ice. Centrifuge at 10000 *x g* and 4°C for 10 min to remove cell debris and save the supernatant. Add 1-50 µl of the Sample supernatant into a 96 well white 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 in which you put 50 µl of NADH Oxidase Assay Buffer.

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: Dilute Lactate Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM Lactate Standard to 990 µl dH_2O, mix well. Dilute 1 mM Lactate Standard solution further to 25 µM Lactate Standard (25 pmol/µl) by adding 10 µl of 1 mM Lactate Standard solution to 390 µl of dH_2O. Add 0, 2, 4, 6, 8, 10 µl of 25 µM Lactate Standard into a series of wells in 96 well white plate to generate 0, 50, 100, 150, 200, 250 pmol/well Lactate Standard. Adjust the volume of all Standard wells to 50 µl with NADH Oxidase Assay Buffer.

3. NADH Oxidase Positive Control: Add 5 µl of reconstituted NADH Oxidase Positive Control into desired well(s) and 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	24 µl	34 µl
NADH Oxidase Enzyme Mix	2 µl	2 µl
NADH Oxidase Substrate I	2 µl	2 µl
NADH Oxidase Substrate II	20 µl	----
NADH Oxidase Developer	----	10 µl
PicoProbe™	2 µl	2 µl

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

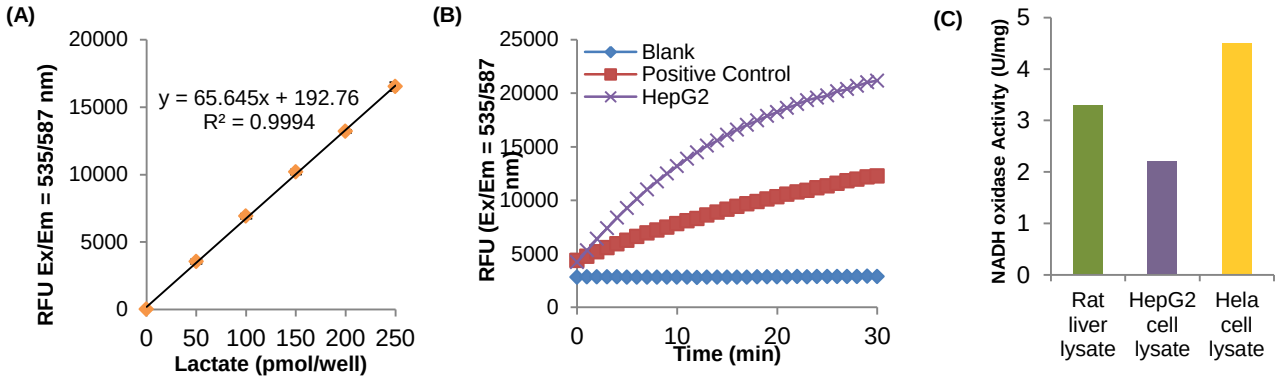
5. Measurement: Measure the fluorescence (Ex/Em = 535/587 nm) in a kinetic mode for 30-40 min at RT.

Note: Incubation time depends on the NADH Oxidase Activity in the Samples. We recommend measuring the fluorescence in a kinetic mode and choosing any two time points (T_1 & T_2) in the linear range to calculate the NADH Activity of the Samples. The Lactate 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. Plot the Lactate Standard Curve. Subtract the Blank readings from the Sample readings. Apply the corrected Sample reading to the Lactate Standard Curve to get B pmol of Lactate generated during the reaction time ($\Delta T = T_2 - T_1$). To determine the activity of NADH Oxidase, use the following equation:

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

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



Figures: (A) Lactate Standard Curve. One mole of Lactate corresponds to one mole of β-NAD+ reduced to NADH, which subsequently generates one mole of reduced substrate. (B) Reaction kinetics of recombinant NADH oxidase (Positive Control) and HepG2 cell lysate. (C) NADH oxidase activity detected in rat liver lysate (1.3 µg total protein), HepG2 cell lysate (6 µg total protein) and Hela cell lysate (0.86 µg total protein). 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 Activity Colorimetric Assay Kit (# K726)
EZScreen™ NAD+/NADH Colorimetric Assay Kit (384-well) (# K958)

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