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ab273345
NADH Oxidase Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273345
(use https://www.abcam.cn/ab273345 for china, or
https://www.abcam.co.jp/ab273345 for Japan)

For the determination of NADH Oxidase activity in adherent/suspension cells and tissue.

This product is for research use only and is not intended for diagnostic use.

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.

Table of Contents

1. Overview

NADH Oxidase Activity Assay Kit (Fluorometric) (ab273345) couple's oxidation of NADH by NADH Oxidase (NOX) and reduction of a Light blue color 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.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix and Standard Mix to the appropriate wells.

Measure fluorescence in kinetic mode for 30-40 mins at RT.

Determine NADH Oxidase activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Assay Buffer XII/NADH Oxidase Assay Buffer	25 mL	-20°C
NADH Oxidase Developer/NADH Oxidase Developer Lyophilized	2 vials	-20°C
Enzyme Mix XV/NADH Oxidase Enzyme mix Lyophilized	1 vial	-20°C
Assay Buffer V/NADH Oxidase Lysis Buffer	25 mL	-20°C
NADH Oxidase Positive Control/NADH Oxidase Positive Control Lyophilized	1 vial	-20°C
NADH Oxidase Substrate I	20 µL	-20°C
NADH Oxidase Substrate II	300 µL	-20°C
L(+)-Lactate Standard/L (+) Lactate Standard	100 µL	-20°C
PicoProbe I/NOX Probe	400 µL	-20°C

7. Materials Required, Not Supplied

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

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm (temperature-controlled)
- 96-well white opaque plate with flat bottom
- dH_2O

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer XII/NADH Oxidase Assay Buffer:**
Ready to use as supplied. Bring to room temperature before use. Store at +4°C.

9.2 **Assay Buffer V/NADH Oxidase Lysis Buffer:**
Ready to use as supplied. Bring to room temperature before use. Store at +4°C.

9.3 **PicoProbe I/NOX Probe:**
Ready to use as supplied. Bring to room temperature before use. Store at -20°C.

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

9.5 **L(+)-Lactate Standard/L (+) Lactate Standard:**
Ready to use as supplied. Store at -20°C. Keep on ice during use.

9.6 **NADH Oxidase Substrate I:**

Dilute with 200 µl Assay Buffer XII/NADH Oxidase Assay Buffer. Store at -20°C. Keep on ice while in use.

9.7 **NADH Oxidase Substrate II:**

Dilute NADH Oxidase Substrate II to 10 mM by adding 300 µl of 100 mM NADH Oxidase Substrate II to 2.7 ml Assay Buffer XII/NADH Oxidase Assay Buffer. Mix well. Aliquot and store at -20°C. Keep on ice while in use.

9.8 **NADH Oxidase Developer/NADH Oxidase Developer Lyophilized:**
Reconstitute each vial with 210 µl Assay Buffer XII/NADH Oxidase Assay Buffer. Store at -20°C. Keep on ice while in use.

9.9 **NADH Oxidase Positive Control/NADH Oxidase Positive Control Lyophilized:**
Reconstitute the vial with 200 µl Assay Buffer XII/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.

10. Sample Preparation

Tissue/cell lysate preparation:

10.1 Homogenize tissue (10 mg) or cells (1 x 10^6) with 200 µl ice cold Assay Buffer V/NADH Oxidase Lysis Buffer on ice.
10.2 Centrifuge at 10,000 *x g* and 4°C for 10 mins to remove cell debris and save the supernatant.
10.3 Add 1-50 µl of the Sample supernatant into a 96 well white plate with flat bottom.
10.4 Bring the volume of all Sample wells to 50 µl with Assay Buffer XII/NADH Oxidase Assay Buffer.
10.5 Prepare one well as Blank well in which you put 50 µl of Assay Buffer XII/NADH Oxidase Assay Buffer.

Δ Note: For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

11. Standard Curve

– Keep standards on ice while in use.

11.1 Dilute L(+)-Lactate Standard/L (+) Lactate Standard to 1mM by adding 10 µl of L(+)-Lactate Standard/L (+) Lactate Standard to 990 µl dH_2O, mix well.

11.2 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.

11.3 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.

11.4 Adjust the volume of all Standard wells to 50 µl with Assay Buffer XII/NADH Oxidase Assay Buffer.

Standard #	25 µM L(+)-Lactate Standard/L (+) Lactate Standard (µL)	Assay Buffer XII/NADH Oxidase Assay Buffer (µL)	L (+) Lactate (pmol/well)
1	0	50	0
2	2	48	50
3	4	46	100
4	6	44	150
5	8	42	200
6	10	40	250

12. Assay Procedure

– Keep on ice while in use.

12.1 **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 Assay Buffer XII/NADH Oxidase Assay Buffer.

12.2 **Reaction Mix:**
Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Reaction mix:

	Reaction mix (µl)	Standard mix (µl)
Assay Buffer XII/NADH Oxidase Assay Buffer	24	34
Enzyme Mix XV/NADH Oxidase Enzyme Mix	2	2
NADH Oxidase Substrate I	2	2
NADH Oxidase Substrate II	20	---
NADH Oxidase Developer	---	10
PicoProbe I/NOX Probe	2	2

12.3 Add 50 µl of the Reaction mix into Positive Control and sample wells.
12.4 Add 50 µl of Standard mix into lactate standard wells.
12.5 Measure fluorescence (Ex/Em = 535/587 nm) in kinetic mode for 30-40 mins at room temperature.

Δ 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 (T1 and T2) 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 mins incubation).

13. Calculations

13.1 Subtract 0 Standard reading from all standard readings.
13.2 Plot the Lactate Standard Curve.
13.3 Subtract the Blank readings from the Sample readings.
13.4 Apply the corrected Sample reading to the Lactate Standard Curve to get B pmol of Lactate generated during the reaction time ($\Delta T = T_2 - T_1$).
13.5 To determine the activity of NADH Oxidase, use the following equation:

$$NOX\ activity\ = \frac{B}{(\Delta T\ x\ P)} = pmol/min/mg = \mu U/mg$$

B = Lactate amount from the Standard Curve (pmol)
ΔT = Reaction time ($T_2 - T_1$) (mins)
P = Amount of protein in sample (mg)

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

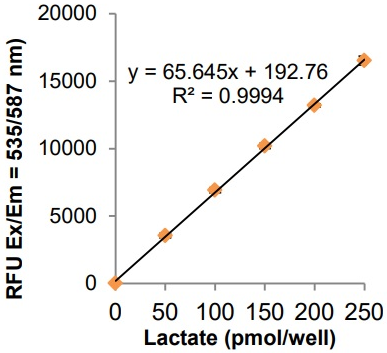

Figure 1. Lactate Standard Curve. One mole of Lactate corresponds to one mole of β-NAD+ reduced to NADH, which subsequently generates one mole of reduced substrate.

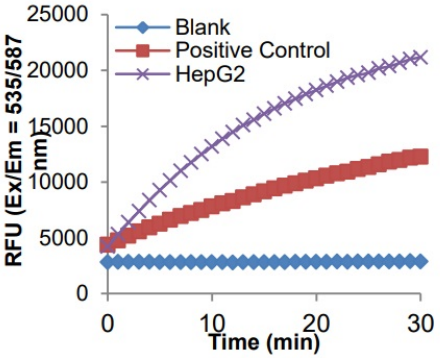

Figure 2. Reaction kinetics of recombinant NADH oxidase (Positive Control) and HepG2 cell lysate.

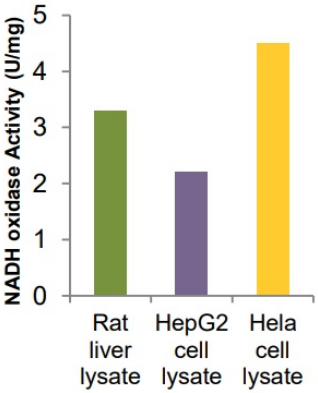

Figure 3. NADH oxidase activity detected in rat liver lysate (1.3 µg total protein), HepG2 cell lysate (6 µg total protein) and HeLa cell lysate (0.86 µg total protein).

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)