ab234049 – NADH Assay Kit (Fluorometric, High Sensitivity)

For the measurement of NADH in a variety of samples including animal tissues, adherent or suspension cells and enzymatic reactions.
For research use only - 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.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab234049

Storage and Stability

- Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Reconstituted components are stable for 2 months.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Materials Supplied

Item	Quantity	Storage Condition
Cycling Buffer I/NADH Cycling Buffer	15 ml	-20°C
NADH Cycling Enzyme Mix/NADH Cycling Enzyme Mix (Lyophilized)	1 vial	-20°C
Extraction Buffer II/NADH Extraction Buffer	50 ml	-20°C
NADH Standard II/NADH Standard (Lyophilized)	1 viall	-20°C
PicoProbe I/PicoProbe™ (in DMSO, ready to use)	0.4 ml	-20°C

Materials Required, Not Supplied

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

- 96-well white plate
- Multi-well spectrophotometer (Fluorescence reader)
- DMSO

Reagent Preparation

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

PicoProbe I/PicoProbe™: Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

NADH Cycling Enzyme Mix: Reconstitute with 220 µl Cycling Buffer I/NADH Cycling Buffer. Pipette up and down to dissolve completely. Aliquot and store at -70°C. Avoid repeated freeze/thaw. Keep on ice while in use.

NADH Standard II/NADH Standard: Reconstitute with 200 µl DMSO to generate 1 mM (1 nmol/µl) NADH Standard II/NADH Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

NADH Assay Protocol

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve must be run with each assay.

Sample Preparation: Liquid samples can be measured directly. Tissue (~10 mg) or cells (~1 x 106) should be rapidly homogenized with 200 µl ice cold Extraction Buffer II/NADH Extraction Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl samples into an eppendorf tube/white plate and bring the volume to 80 µl with Extraction Buffer II/NADH Extraction Buffer.

∆ **Notes:**

a) For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the standard curve range.
b) Cell or tissue lysates may contain enzymes that consume NADH rapidly. We suggest removing these enzymes by filtering the samples through 10 Kd molecular weight cut off filters before performing the assay.

NAD Decomposition: To detect NADH, the NAD needs to be decomposed before the reaction. Put samples at 600C for 30 minutes to completely decompose the NAD. Cool samples on ice. Centrifuge briefly and transfer 50 µl of samples into a 96 well white plate.

Standard Curve Preparation: Dilute NADH to 10 pmol/µl by adding 10 µl of 1 mM NADH to 990 µl dH2O, mix well. Dilute further to 0.1 pmol/µl by adding 10 µl of 10 pmol/µl NADH into 990 µl dH2O, mix well. Add 0, 4, 8, 12, 16 & 20 µl of diluted 0.1 pmol/µl NADH Standard II/NADH Standard into a 96 well plate to generate 0, 0.4, 0.8, 1.2, 1.6, and 2 pmol/well NADH Standards. Adjust the volume to 50 µl/well with Extraction Buffer II/NADH Extraction Buffer.

Reaction Mix: Mix enough reagents for the number of assays (samples and standards) to be performed. For each well, prepare 100 µl Reaction Mix containing:

Item	Reaction Mix	Background Control Mix
Cycling Buffer I/NADH Cycling Buffer	96 µl	98 µl
NADH Cycling Enzyme Mix	2 µl	-
PicoProbe I/PicoProbe™	2 µl	2 µl

Add 100µl of the Reaction Mix to each well containing the Standard & test samples, mix well.
∆ **Note:** If your sample has fluorescence background, prepare a parallel sample well as the background control.

Measurement

Incubate the reaction for 30 min. at room temperature. Measure fluorescence (Ex/Em = 535/587 nm).

Calculation

Subtract the 0 NADH Standard reading from all readings. Plot the NADH Standard curve. If the sample background control reading is significant, subtract the background control reading from the sample. Apply the corrected sample reading to the Standard Curve to get B pmol of NADH in the sample well.

Sample NADH concentration (C) = B/V x Dilution Factor = pmol/µl = nmol/ml

Where: **B** = Amount of NADH in the sample well (pmol).
V = Sample volume used in the reaction well (µl).

NADH in Samples can also be expressed in pmol/mg of sample.
NADH molecular weight: 663.43 g/mole.

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)

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