

ab282929 NAD⁺/NADH Colorimetric Assay Kit

For the measurement of NAD, NADH or their ratio in various tissues/cell extracts and biological fluids and for the analysis of metabolism in various cells.
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/ab282929>

Storage and Stability: Entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

| Item | Quantity | Storage Condition |
|--|----------|-------------------|
| Extraction Buffer II /NADH/NAD Extraction Buffer | 50 mL | -20°C |
| Cycling Buffer I/NAD Cycling Buffer | 15 mL | -20°C |
| NAD Cycling Enzyme Mix | 1 vial | -20°C |
| Developer Solution II/NADH Developer | 1 vial | -20°C |
| Stop Solution II/Stop Solution | 1.2 ml | -20°C |
| NADH Standard II/NADH Standard | 1 vial | -20°C |

Materials Required, Not Supplied

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

- 384-well clear plate with flat bottom
- Multi-well spectrophotometer with 384-well plate reading capability

Reagent Preparation Before using the kit, spin the tubes prior to opening.

Extraction Buffer II / NADH/NAD Extraction Buffer and Cycling Buffer I/NAD Cycling Buffer: Warm both buffers to room temperature before use. Store at -20°C.

NAD Cycling Enzyme Mix: Reconstitute with 220 µl Cycling Buffer I/NAD Cycling Buffer. Aliquot the reconstituted

Cyclic Enzyme in Eppendorf tubes and freeze immediately at -70°C for future use. The enzyme is stable for up to 2 months at -70°C after reconstitution. Keep the aliquoted enzyme vial in ice when setting the assay-experiment.

Developer Solution II/NADH Developer: Reconstitute Developer Solution II/NADH developer with 1.2 ml of ddH₂O. Pipette up and down several times to completely dissolve the pellet into solution (don't vortex). Store at -20°C, protected from light. Use within 2 months.

NADH Standard II/NADH Standard: Reconstitute with 200 µl pure DMSO to generate 1 nmol/µl NADH Standard II/NADH Standard. Store at -20°C. Use within 2 months.

Assay Protocol

Sample Preparation

For cells: wash cells with cold PBS. Pellet 2 X 10⁵ cells for each assay in a micro-centrifuge tube (2000 rpm, 5 min, 4 °C) & extract with 400 µl of Extraction Buffer II/NADH/NAD Extraction Buffer by freeze/thaw two cycles (20 min. on dry-ice, then 10 min. at room temperature), or by homogenization. Centrifuge at 4 °C for 18000 x g, 10 min. Transfer the extracted NADH/NAD supernatant into a new tube.

For tissues: Weigh ~20 mg tissue & wash with cold PBS. Homogenize in 200 µl of Extraction Buffer II /NADH/NAD Extraction Buffer in new tube. Centrifuge at 4°C 18000xg, 10 min. Transfer the supernatant into a fresh tube.

For serum and urine: centrifuge the samples at 4 °C for 18000 x g, 10 min to remove any particles. Collect supernatant.

To detect total NAD (NAD⁺: NADH and NAD): Transfer 5 µl of samples into a 384-well clear plate. Make the final volume to 10 µl with Extraction Buffer II / NADH/NAD extraction buffer.

To detect NADH: NAD needs to be decomposed before reacting with NAD Cycling Enzyme Mix. To decompose NAD keeping NADH intact, aliquot 100 µl of extracted samples into Eppendorf tubes and incubate (60°C; 30 min). All NAD will decompose, while NADH will still be intact. Cool samples on ice. Do a quick spin of the samples at 4 °C for 18000 x g, 1 min., transfer 5 µl of NAD-decomposed samples into a 384-well clear plate. Make the final volume to 10 µl with Extraction Buffer II / NADH/NAD extraction buffer.

Notes:

- Cell or tissue lysates and biological fluid samples may contain enzymes that consume NADH rapidly. We suggest removing these enzymes by filtering the samples through 10 kDa molecular weight cut off filters before performing the assay.
- For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions with the Extraction Buffer II/extraction buffer to ensure the readings are within the Standard Curve range.
- For samples having high background, prepare parallel well(s) containing same amount of sample as in the test well. Adjust the volume to 10 µl/well with Extraction Buffer II / NADH/NA Extraction Buffer.
- Endogenous compounds may interfere with the reaction. To ensure accurate determination of NADH in the test samples, we recommend spiking samples with 10 pmol of Standard.
- Instrument reader settings need to be adjusted according to the chosen 384-well plate clear plate.

Standard Curve Preparation

- Dilute 2.5 µl of 1 nmol/µl NADH Standard II/NADH standard with 997.5 µl Extraction Buffer II / NADH/NAD Extraction Buffer to generate 2.5 pmol/µl standard NADH.
- Add 0, 2, 4, 6, 8 and 10 µl of the diluted NADH standard into 384-well plate to generate 0, 5, 10, 15, 20 and 25 pmol/well standards. Make the final volume to 10 µl with Extraction Buffer II/NADH/NAD extraction buffer.

Note: Diluted NADH solution is unstable, must be used within 4 hours

Reaction Mix

Prepare a Reaction Mix with Cycling Buffer I/NAD Cycling Buffer, NAD Enzyme Mix and Developer Solution II/NADH Developer. For each reaction:

| Item | Reaction Mix | Background Control Mix* |
|---|--------------|-------------------------|
| Cycling Buffer I/NAD Cycling Buffer | 20 μ l | 20.5 μ l |
| NAD Cycling Enzyme Mix | 0.5 μ l | ---- |
| Developer Solution II/NADH Developer | 2.5 μ l | 2.5 μ l |

Mix well and add 23 μ l of the mix into each well of NADH Standard II/NADH Standard and samples.

* For samples having high background, add 23 μ l of Background Control Mix to sample background control well(s).

Measurement: Let the reaction cycling at room temperature for 1 to 4 hrs or longer depending on the absorbance reading at 450 nm. The plate can be read multiple times while the color is being developed. The reactions can be stopped by adding 2 μ l of Stop Solution into each well. After addition of Stop Solution, the color should be stable for 48 h. in a sealed plate.

Calculations:

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

$$\text{Sample NADH concentration (C)} = B/V \times D \text{ pmol}/\mu\text{l}$$

Where:

B is the amount of NADH in the sample well (pmol)

V is the sample volume added into the reaction well (μl)

D is the sample dilution factor

NADH Molecular Weight: 663.4

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, **NAD⁺** or **NADH** amount in well =

$$\frac{(OD_{\text{sample (corrected)}})}{(OD_{\text{sample}} + NADH \text{ Std (corrected)}) - (OD_{\text{sample (corrected)}})} + NADH \text{ Spike (pmol)}$$

NAD/NADH Ratio is calculated as: $(NAD^+ - NADH) / (NADH)$

Technical Support

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