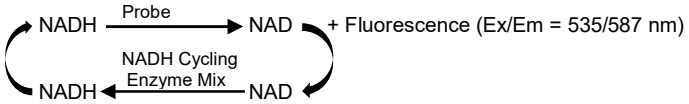
NADH Fluorometric Assay Kit (BN00604)

(Catalog BN00604; 100 assays; Store at -20°C)

I. Introduction:

NAD and NADH exist in all living organisms and are important for energy transformation and maintenance of the redox state of cells or tissues. The reduced form, NADH is a driving force for cells and plays a critical role in cell regulation and repair processes. It is of increasing interest to be able to measure low level of NADH in samples or in enzymatic reactions. Assay Genie's NADH Assay kit provides a highly sensitive method to detect low level of NADH (NAD is not detected in the reaction). There is no requirement to purify NADH from samples. The NADH Recycling Enzyme Mix specifically recognizes NADH (not NADPH) in the enzyme recycling reaction. The assay is rapid, simple and can measure less than 8 nM NADH in a variety of samples.



II. Application:

- Measurement of NADH in various tissues/cells
- Measurement of low levels of NADH in enzymatic reactions
- Analysis of metabolism and cell signaling in various cells

III. Sample Type:

- Animal tissues: Liver, muscle, heart etc.
- Cell culture: Adherent or suspension cells
- Enzymatic reactions

IV. Kit Contents:

Components	BN00604	Cap Code
NADH Extraction Buffer	50 ml	NM
NADH Cycling Buffer	15 ml	NM
Probe (DMSO)	0.4 ml	Blue
NADH Cycling Enzyme Mix (Lyophilized)	1 vial	Green
NADH Standard (Lyophilized)	1 vial	Yellow

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm NADH Extraction Buffer & NADH Cycling Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **Probe:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **NADH Cycling Enzyme Mix**: Reconstitute with 220 µl 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:** Reconstitute with 200 µl DMSO to generate 1 mM (1 nmol/µl) NADH Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

VIII. NADH Assay Protocol:

1. **Sample Preparation:** Liquid samples can be measured directly. Tissue (~10 mg) or cells (~1 x 10^6) should be rapidly homogenized with 200 µl ice cold NADH Extraction Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add1-50 µl samples into an eppendorf tube/white plate and bring the volume to 80 µl with 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 performing the assay.

2. **NAD Decomposition:** To detect NADH, the NAD needs to be decomposed before the reaction. Put samples at 60^0C 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.

3. **Standard Curve Preparation:** Dilute NADH to 10 pmol/µl by adding 10 µl of 1 mM NADH to 990 µl dH_2O, mix well. Dilute further to 0.1 pmol/µl by adding 10 µl of 10 pmol/µl NADH into 990 µl dH_2O, mix well. Add 0, 4, 8, 12, 16 & 20 µl of diluted 0.1 pmol/µl 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 NADH Extraction Buffer.

4. **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:

	Reaction Mix	*Background Control Mix
NADH Cycling Buffer	96 µl	98 µl
NADH Cycling Enzyme Mix	2 µl	--
Probe	2 µl	2 µl

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

5. **Measurement:** Incubate the reaction for 30 min. at room temperature. Measure fluorescence (Ex/Em = 535/587 nm).
6. **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** is the amount of NADH in the sample well (pmol).

V is the 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.

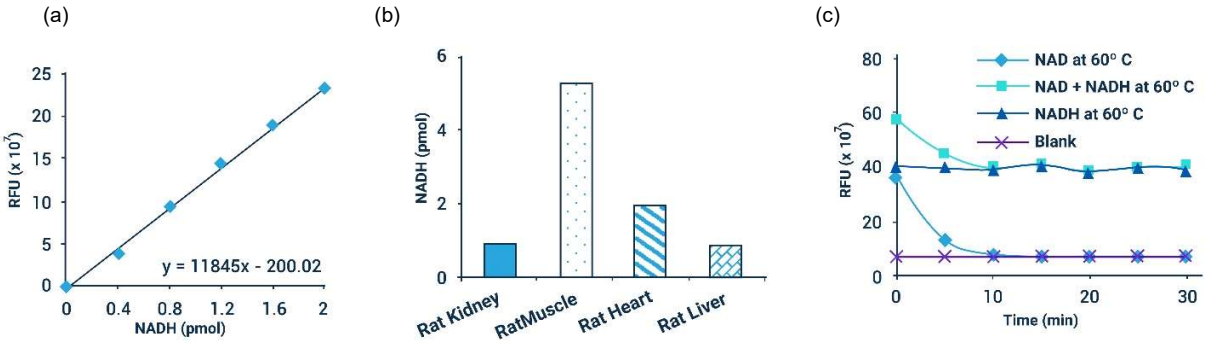


Figure: NADH Standard Curve (a). Decomposition of NAD, but not NADH (b) & measurement of NADH in Rat kidney (1.74 µg), muscle, (2.51 µg), heart (1.59 µg) and liver lysates (29.5 µg) (c).

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