PRODUCT INFORMATION & MANUAL

NAD+/NADH Assay Kit (Colorimetric)

NBP3-25866

For research use only.
Not for diagnostic or therapeutic procedures.

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NAD+/NADH Assay Kit (Colorimetric)

Catalog No: NBP3-25866

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.02 μmol/L

Detection range: 0.02-5.0 μmol/L

Average intra-assay CV (%): 1.8

Average inter-assay CV (%): 9.1

Average recovery rate (%): 90

▲ This kit is for research use only.

▲ Instructions should be followed strictly, changes of operation may result in unreliable results.

▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure NAD^+, NADH content and their ratio in animal tissue and cell samples.

▲Detection principle

NAD^+ and NADH are coenzymes that transfer electrons during REDOX reactions, and can be used as cofactors of many enzymes to participate in intracellular reactions.

Detect total content of NAD^+ and NADH:

Ethanol generates acetaldehyde under the action of enzyme. Meanwhile, NAD^+ is reduced to NADH, NADH, under the action of hydrogen transmitter, transfer electrons to WST-8 to produce the yellow product, which has a characteristic absorption peak at 450 nm. Therefore, the total content of NAD^+ and NADH can be quantified by measure the OD value at 450 nm.

Detect NADH:

After treating sample, heat at 60℃ water bath for 30 min. the NAD^+ of the sample is decomposed and only NADH remains. NADH reduces WST-8 to form yellow product, and the amount of NADH is determined by measure the OD value at 450 nm.

Detect NAD^+ and NAD^+/NADH:

The content of NAD^+ and the ratio of NAD^+/NADH in the sample can be obtained according to the total content of NAD^+ and NADH obtained of the first two steps as well as the separate content of NADH.

Note: $NADP^+$ and NADPH have no effect on the determination results.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	-20℃ , 12 months
Reagent 2	Buffer Solution	16 mL × 1 vial	-20℃ , 12 months
Reagent 3	Chromogenic Agent	5 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 4	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 5	Standard	Powder × 2 vials	-20℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader (450 nm), Micropipettor, 37 ℃ water bath, 10 KD ultrafiltration tube.

Reagents:

PBS (0.01 M, pH 7.4).

⚠Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. Place the prepared reagent 4 working solution at 2-8℃ with shading light for 5 h before use, prepare in advance.
2. The sample must be fresh.
3. Heat the prepared sample at 60℃ water bath for 30 minutes, during this process, the EP tube should be sealed to prevent liquid volatilization. After heating, due to condensation of water vapor, it is necessary to mix thoroughly before proceeding to the next step.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 4 working solution:

   Dissolve a vial of reagent 4 powder with 200 μL of double distilled water and mix fully. Place at 2-8°C with shading light for 5 h before use. The prepared solution can be stored at 2-8°C for 7 days with shading light.
3. Preparation of reaction working solution:

   Mix the reagent 4 working solution and reagent 2 at the ratio of 1: 39 fully. Prepare the fresh solution before use and store with shading light and the prepared solution should be used in 2 h.
4. Preparation of 250 μmol/L standard:

   Dissolve a vial of reagent 5 fully with 200 μL of double distilled water. Prepare the fresh solution before use. The prepared solution can be stored at -20°C for 7 days with shading light.

   Preparation of 5 μmol/L standard:

   Dilute 250 μmol/L standard solution with reagent 1 at the ratio of 1:49. Prepare the fresh solution before use and store with shading light and the prepared solution should be used in 1 day.

▲ Sample preparation

1. Sample homogenate:

Tissue sample:

Weigh 0.02-1g fresh tissue. Homogenize at the ratio of the volume of reagent 1 (mL): the weight of the tissue (g) =9:1, then centrifuge at 10000 g at 4℃ for 10 min. Take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number ($1.5*10^6$): reagent 1 (mL) =1: 0.4. Sonicate or mechanical homogenate in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

2. Sample ultrafiltration:

Tissue and cell homogenate contains enzymes that can decompose NAD^+. It is recommended that after sample extraction and centrifugation, the supernatant be centrifuged with 10 KD ultrafiltration tube at 10000 g for 10 min at 4℃ to remove the catabolase.

Note: Measure total of NAD^+ and NADH: Detect the filtered sample supernatant directly. Measure NADH: Take amount of filtered sample supernatant into EP tube, heat at 60 ℃ for 30 min, and cool with running water for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.02-5.0 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
10% Mouse muscle tissue homogenate	1
10% Mouse kidney tissue homogenate	1
293T cell	1
Hela cell	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 5 μmol/L standard with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0.0, 1, 1.5, 2, 2.5, 3.5, 4, 5 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	5 μmol/L standard (μL)	Reagent 1 (μL)
A	0	0	200
B	1	40	160
C	1.5	60	140
D	2	80	120
E	2.5	100	100
F	3.5	140	60
G	4	160	40
H	5	200	0

2. The measurement of samples

(1) Sample well: Take 20 μL of sample supernatant into corresponding sample wells.

Standard well: Take 20 μL of standard solution with different concentrations into corresponding standard wells.

(2) Take 120 μL of reaction working solution into each well.

(3) Add 40 μL of reagent 3 to each well.

(4) Mix fully with microplate reader for 5 s and incubate at 37°C for 30 min. Measure the OD value of each well at 450 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Sample supernatant (μL)		20
Standard of different concentrations (μL)	20	
Reaction working solution (μL)	120	120
Regent 3 (μL)	40	40
Mix fully and incubate at 37℃ for 30 min. Measure the OD value of each well.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. For total content of NAD^+ and NADH:

$$[NAD]_{total}\,(\mu mol/gprot) = (\Delta A - b) \div a \times f \div C_{pr}$$

2. For NADH:

$$[NADH]\,(\mu mol/gprot) = (\Delta A - b) \div a \times f \div C_{pr}$$

3. For NAD^+:

$$[NAD^+]\,(\mu mol/gprot) = [NAD]_{total} - [NADH]$$

4. For NAD^+/NADH:

$$[NAD^+] / [NADH] = ([NAD]_{total} - [NADH])/ [NADH] \times 100\%$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before tested.

ΔA: $OD_{Sample} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

C_{pr}: Concentration of protein in sample supernatant before filter, gprot/L.

Appendix I Data

▲Example analysis

For mouse muscle tissue, take 20 μL of 10% mouse muscle tissue homogenate, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.2771 x + 0.0107, the average OD value of the blank is 0.158, the average OD value of the sample for NAD_{total} is 0.565, the average OD value of the sample for NADH is 0.466, the concentration of protein in sample is 2.80 gprot/L, and the calculation result is:

$$[NAD]_{total}\ (\mu mol/gprot) = (0.565 - 0.158 - 0.0107) \div 0.2771 \div 2.80$$

$$= 0.510\ \mu mol/gprot$$

$$[NADH]\ (\mu mol/gprot) = (0.466 - 0.158 - 0.0107) \div 0.2771 \div 2.80$$

$$= 0.383\ \mu mol/gprot$$

$$[NAD^+]\ (\mu mol/gprot) = 0.510 - 0.383 = 0.127\ \mu mol/gprot$$

$$[NAD^+] / [NADH] = (0.510 - 0.383) \div 0.383 \times 100\% = 33.2\ \%$$