PRODUCT INFORMATION & MANUAL

NADP+/NADPH Assay Kit (Colorimetric)

NBP3-25887

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

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NADP+/NADPH Assay Kit (Colorimetric)

Catalog No: NBP3-25887

Method: Colorimetric method

Specification: 96T (Can can detect 80 samples for total content of $NADP^+$ and NADPH without duplication, while detect 40 samples for $NADP^+$ or $NADP^+$/NADPH)

Measuring instrument: Microplate reader

Sensitivity: 0.02 μmol/L

Detection range: 0.02-5.0 μmol/L

Average intra-assay CV (%): 2.1

Average inter-assay CV (%): 5.5

- 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 NADP+, NADPH content and their ratio in animal tissue and cells samples.

▲Background

NADP (Nicotinamide adenine dinucleotide phosphate) is a coenzyme of many REDOX reactions, including $NADP^+$ (oxidized form) and NADPH (reduced form). $NADP^+$ is also involved in biosynthetic reactions such as the synthesis of lipids and nucleic acids. In animal cells, the oxidation phase of the pentose phosphate pathway is the most important source of NADPH.

▲Detection principle

Detect total content of $NADP^+$ and NADPH:

Glucose 6-phosphate (G6P) is oxidized to 6-phosphate gluconolactone (6-PG) by glucose-6-phosphate dehydrogenase (G6PDH), and $NADP^+$ is reduced to NADPH during this reaction. NADPH, under the action of 1-mPMS, transfer electrons to WST-8 to produce the yellow product, which has a characteristic absorption peak at 450 nm. Therefore, the total content of $NADP^+$ and NADPH can be quantified by measure the OD value at 450 nm.

Detect NADPH:

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

Detect $NADP^+$ and $NADP^+$/NADPH:

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

Note: NAD^+ and NADH 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	12 mL × 1 vial	-20℃ , 12 months
Reagent 3	Chromogenic Agent	1.2 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months
Reagent 5	NADPH Standard	Powder × 1 vial	-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

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

Reagents:

Ultrapure water

⚠ 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. The sample must be fresh.
2. After heat the prepared sample in a water bath at 60℃ for 30 minutes, if there is turbidity, centrifuge at 10000 g at 4℃ for 10 minutes and then take the supernatant for detection.

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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 powder with 0.12 mL of ultrapure water and mix fully. Prepare the fresh solution before use. Aliquot the prepared solution into small quantities and it can be stored at -20℃ for 2 days with shading light.

   Preparation of reaction working solution:

   Mix the reagent 4 working solution and reagent 2 at the ratio of 1: 49 fully. Prepare the fresh solution before use and store with shading light.
3. Preparation of 1 mmol/L NAPDH Standard:

   Dissolve a vial of reagent 5 fully with 4.8 mL of ultrapure water. Prepare the fresh solution before use. Aliquot the prepared solution into small quantities and it can be stored at -20℃ for 7 days with shading light.
4. Preparation of 10 μmol/L NAPDH Standard solution:

   Dilute 1 mmol/L standard with reagent 1 at the ratio of 1:99. Prepare the fresh solution before use.

▲ Sample preparation

1. Cell sample:

1) For adherent cells, discard the culture medium, wash the cells with precooled PBS (0.01 mol/L, pH 7.4). Scratch the cells with cell scraper, add 2-5 mL of precooled PBS (0.01 mol/L,pH 7.4), centrifuge at 1000 g at 4℃ for 10 min. Collect cells and add precooled reagent 1 at a ratio of cell number ($4*10^6$): reagent 1 (mL) =1: 0.8. Blow and beat gently, stand for 10 min to lyse cells.

2) For suspension cells, take about $4*10^6$ cells, centrifuge at 600 g at 4℃ for 5 min, discard the supernatant. Add 0.8 mL of pre-cooled reagent 1, blow and beat gently, stand for 10 min to lyse cells.

Note: The lysing process can be operated at room temperature or on ice. Centrifuge at 12000 g at 4℃ for 10 min, and take the supernatant for detection.

2. 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 12000 g at 4℃ for 10 min. Take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Note: Reagent 1 contains enzymes that can decompose NADPH. It is recommended that after sample extraction and centrifugation, filter the supernatant through a 10 kD ultrafiltration tube to remove the enzymes.

▲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
Jurkat cells	1
Mark cells	1
HCT116 cells	1
293T cells	1
10% Mouse kidney tissue homogenate	1
Hela cells	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 operating steps

The preparation of standard curve

Dilute 10 μmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	10 μmol/L Standard (μL)	Reagent 1 (μL)
A	0	0	500
B	1.0	50	450
C	1.5	75	425
D	2.0	100	400
E	2.5	125	375
F	3.0	150	350
G	4.0	200	300
H	5.0	250	250

The pretreatment of sample

Measure total of $NADP^+$ and NADPH: Detect the filtered sample supernatant directly.

Measure NADPH: Take 0.2 mL of filtered sample supernatant into EP tube, heat at 60 ℃ for 30 min, and cool with running water for detection.

The measurement of samples

1) Sample well: Take 50 μL of sample supernatant into corresponding sample wells.
   Standard well: Take 50 μL of standard solution with different concentrations into corresponding standard wells
2) Take 100 μL of reaction working solution into each well.
3) Mix fully with microplate reader for 5 s and incubate at 37℃ for 10 min.
4) After the incubation, add 20 μL of reagent 3 to each well immediately.
5) Mix fully with microplate reader for 5 s and incubate at 37℃ for 10 min. Measure the OD value of each well at 450 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Sample supernatant (μL)		50
Standard of different concentrations (μL)	50	
Reaction working solution (μL)	100	100
Mix fully and incubate at 37℃ for 10 min.		
Reagent 3 (μL)	20	20
Mix fully and incubate at 37℃ for 10 min. Measure the OD value at 450 nm.		

▲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 NADP+ and NADPH:

$$[NADP]_{total}\,(\mu mol/gprot) = (\Delta A_1 - b) \div a \times f \div C_{pr}$$

2. For NADPH:

$$[NADPH]\,(\mu mol/gprot) = (\Delta A_2 - b) \div a \times f \div C_{pr}$$

3. For $NADP^+$:

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

4. For $NADP^+$/NADPH:

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

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 test.

ΔA_1: $OD_{Sample} - OD_{Blank}$ (for total content of $NADP^+$ and NADPH).

ΔA_2: $OD_{Sample} - OD_{Blank}$ (for NADPH).

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

Appendix I Data

▲Example analysis

For Jurkat cell, take 50 μL of prepared cell supernatant into corresponding wells and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.3807 x – 0.0232, the average OD value of the blank is 0.075, the average OD value of the sample for $NADP_{total}$ is 0.654, the average OD value of the sample for NADPH is 0.400, the concentration of protein in sample is 0.063 gprot/L and the calculation result is:

$[NADP]_{total}$ (μmol/gprot) = (0.654-0.075 +0.0232) ÷ 0.3807 ÷0.063

=25.11μmol/gprot

[NADPH] (μmol/gprot) = (0.400-0.075 +0.0232) ÷ 0.3807 ÷0.063

=14.52 μmol/gprot

$[NADP^+]$ (μmol/gprot) = 25.11- 14.52 =10.59 μmol/gprot

$[NADP^+]$ / [NADPH] = 10.59 ÷ 25.11 ×100% =42.3%