（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K803-M

Specification：96T(80 samples)

Measuring instrument：Microplate reader（450 nm）

Detection range：0.02-5.0 μmol/L

Elabscience®$NADP^+$/NADPH Colorimetric Assay Kit (WST-8)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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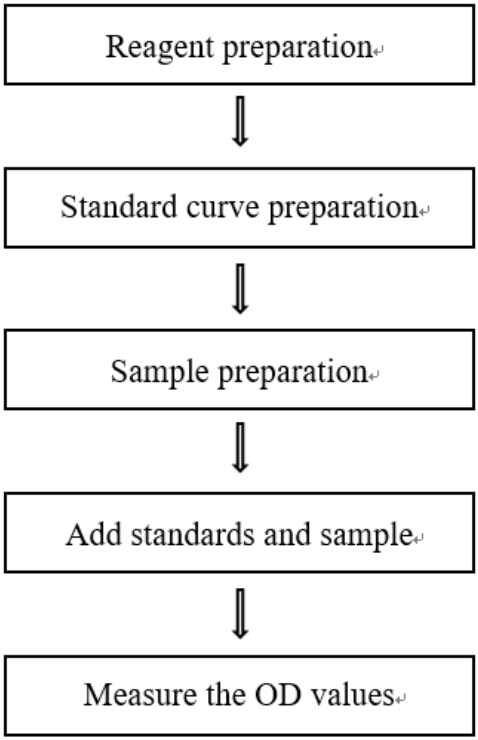
Assay summary



Intended use

This kit can be used to measure $NADP^+$, NADPH content and their ratio in animal tissue and cells samples.

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	Size (96 T)	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	
	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Ultrapure water, PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of enzyme working solution：

Dissolve one vial of enzyme reagent with 120 μL of ultrapure water, mix well to dissolve. The enzyme working solution should be prepared on spot. Store at -20 ℃ for 2 days protected from light.

③ Preparation of reaction working solution：

For each well， prepare 100 μL of reaction working solution (mix well 2 μL of enzyme working solution and 98 μL of buffer solution). The reaction working solution should be prepared on spot and stored protected from light.

④ Preparation of 1 mmol/L NAPDH Standard：

Dissolve one vial of NADPH standard with 4.8 mL of ultrapure water, mix well to dissolve. The 1 mmol/L NAPDH standard should be prepared on spot. Aliquoted storage at -20 ℃ for 7 days protected from light.

⑤ Preparation of 10 μmol/L NAPDH standard solution：

Before testing, please prepare sufficient 10 μmol/L NAPDH standard solution according to the test wells. For example, prepare 1000 μL of 10 μmol/L NAPDH standard solution (mix well 10 μL of 1 mmol/L NAPDH standard and 990 μL of extracting solution). The 10 μmol/L NAPDH standard solution should be prepared on spot.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 10 μmol/L standard solution with extracting solution 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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**4.0**	**5.0**
10 μmol/L standard (μL)	0	50	75	100	125	150	200	250
Extracting solution (μL)	500	450	425	400	375	350	300	250

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 50 mg tissue in 450 μL extracting solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Note: Homogenized medium 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.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 4×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 4×10^6 cells in 800 μL extracting solution, Blow and beat gently, stand for 10 min to lyse cells..

④ Centrifuge at 12000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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 extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

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.

Operating steps

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 sample

① Standard well: Take 50 μL of standard solution with different concentrations into corresponding standard wells.
Sample well: Take 50 μL of sample supernatant into corresponding sample 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 chromogenic agent 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. For total content of NADP+ and NADPH:

$$[NADP]total\ (\mu mol/gprot) = (\Delta A1 - b) \div a \times f \div C_{pr}$$

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]

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 Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	0.42	2.60	4.30
%CV	2.4	2.2	1.7

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	0.42	2.60	4.30
%CV	5.3	5.1	6.1

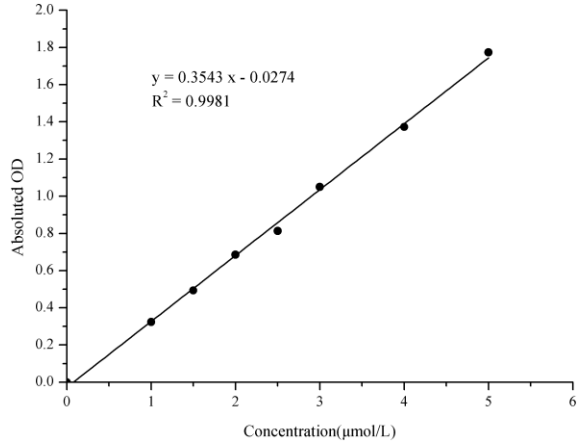
Sensitivity

The analytical sensitivity of the assay is 0.02 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**4.0**	**5.0**
Average OD	0.081	0.405	0.574	0.767	0.895	1.132	1.454	1.855
Absoluted OD	0.000	0.324	0.493	0.686	0.814	1.051	1.373	1.774



Appendix II Example Analysis

Example analysis:

For Jurkat cell, take 50 μL of prepared cell supernatant into corresponding wells and carry the assay according to the operation steps. 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:

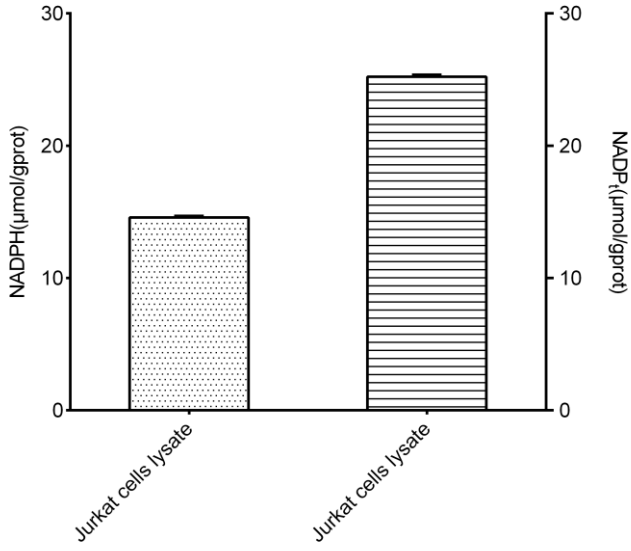
$$[NADP]_{total}\ (\mu mol/gprot) = (0.654 - 0.075 + 0.0232) \div 0.3807 \div 0.063 = 25.11 \mu mol/gprot$$

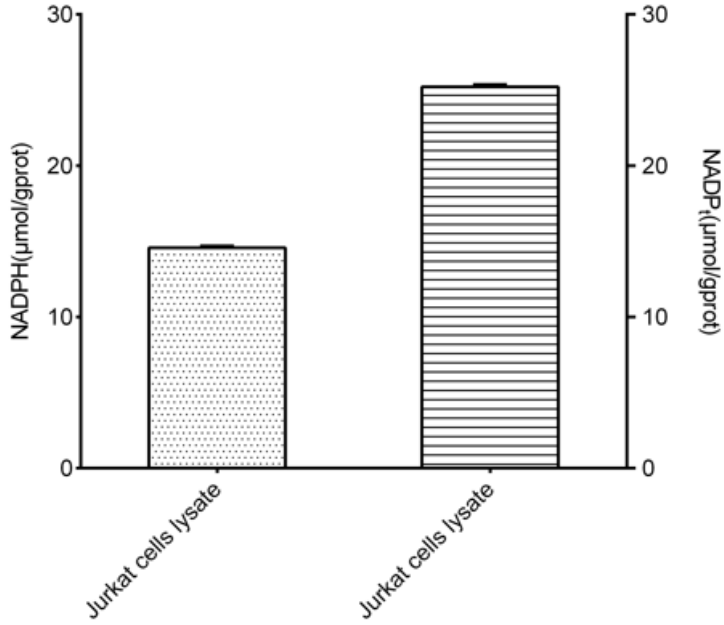
$$[NADPH]\ (\mu mol/gprot) = (0.400 - 0.075 + 0.0232) \div 0.3807 \div 0.063 = 14.52\ \mu mol/gprot$$

$$[NADP^+]\ (\mu mol/gprot) = 25.11 - 14.52 = 10.59\ \mu mol/gprot$$

$$[NADP^+] / [NADPH] = 10.59 \div 25.11 \times 100\% = 42.3\%$$

Detect Jurkat cells (the concentration of protein is 0.063 gprot/L) and HCT116 cells (the concentration of protein is 0.068 gprot/L) according to the protocol, the result is as follows:




30
20
10
0
NADPH(μmol/gprot)
30
20
10
0
NADPH(μmol/gprot)
Jurkat cells lysate
Jurkat cells lysate

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.