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NADP+/NADPH Assay Kit (WST-8 Method)

Catalog No.：BC00030

Size：100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email（Sale） order@enkilife.com

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	NADP+/NADPH Assay Kit (WST-8 Method)
Detection Methods	Colorimetric
Sample type	Tissue, cell and other samples
Detection Type	Quantitative
Detection instrument and wavelength	Microplate reader (450 nm)

Product Introduction

This kit is a colorimetric kit based on WST-8, which detects the amount, ratio and total amount of NAD + (oxidized coenzyme I) and NADH (reduced coenzyme I) in cells, tissues or other samples by colorimetry. NAD (Nicotinamide adenine dinucleotide) is a coenzyme present in all cells, including two forms: NAD^+ (oxidized form) and NADH (reduced form) . NAD^+ is not only a coenzyme that transfers electrons during redox reactions, but also can be used as a substrate for many enzymes to participate in intracellular reactions. For example, deacetylases such as Sirt1 of the Sirtuins family need NAD^+ as a substrate for deacetylation reactions to regulate the acetylation level of proteins and thus participate in the life activities of cells. NAD^+ plays an important role in cells and the body. Its synthesis and degradation and its products are involved in cell apoptosis, metabolic regulation and gene expression regulation, and the reduction of NAD^+ is one of the main factors for cell death. The importance of NAD^+ in regulating the cellular redox state and its function in regulating signaling pathways and transcription make NAD^+ and the enzymes that synthesize and consume it potential drug targets for a variety of diseases.

Features

★ WST-8 is an upgraded substitute for MTT, and has obvious advantages over MTT or other MTT-like products such as XTT, MTS, etc. First, the formazan generated by the reduction of MTT by some dehydrogenases is not water-soluble and requires a specific dissolving solution to dissolve; while the formazan generated by WST-8, XTT, and MTS are all water-soluble, which can save the subsequent dissolution step. Secondly, the formazan generated by WST-8 is more soluble than the formazan generated by XTT and MTS. Thirdly, WST-8 is more stable than XTT and MTS, making the experimental results more stable.

★ Compared with MTT, XTT, etc., WST-8 has a wider linear range and higher sensitivity.

★ Compared with WST-1, WST-8 has higher detection sensitivity, is more soluble, and is more stable.

★ This kit is easy to use, highly sensitive, and has a wide linear range. It can detect NAD^+ or NADP as low as 0.25 μM , and shows a good linear relationship between 0.25 μM and 10 μM. The detection can be performed using lysate of cells, tissues, etc., without the need to separate and purify NAD^+ and NADH in cells, tissues, or other samples, and can specifically detect NAD^+ and NADH, but not $NADP^+$ and NADPH.

Detection principle

The traditional detection method of NAD^+/NADH is to detect the change of NADH absorption wavelength at 340nm . This method has low sensitivity and is easily interfered by similar ultraviolet absorbing substances in the sample. In addition, it is usually necessary to increase the amount of sample to compensate for the deficiency of too low absorbance of NADH at 340nm during ultraviolet detection . Therefore, this traditional detection method has great limitations. This kit can detect the content of NAD^+ and NADH in the sample and their ratio. The specific principle is as follows:

a. Determination of the total amount of NAD^+ and NADH: Ethanol is oxidized to acetaldehyde by alcohol dehydrogenase (ADH), and NAD^+ is reduced to NADH in this reaction process; the generated NADH reduces WST-8 to orange-yellow formazan by the action of the electron coupling reagent 1-mPMS (1-Methoxy-5-methylphenazinium Methyl Sulfate), with a maximum absorption peak at around 450nm. The formazan generated in the reaction system is proportional to the total amount of NAD^+ and NADH in the sample.

b. Determine the amount of NADH alone: After heating in a 60ºC water bath for 30 minutes, the NAD+ in the sample will decompose and only NADH will remain. NADH reduces WST-8 to formazan, and the amount of formazan generated by the reaction is determined by colorimetry, and ultimately the amount of NADH in the sample can be determined.

c. Determination of NAD^+ and NAD^+/NADH ratio: Based on the total amount of NAD^+ and NADH and the amount of NADH obtained in the first two steps, the amount of NAD + in the sample and the ratio of NAD^+/NADH can be calculated .

Product composition

Serial Number	Product Name	Packing Specifications	Storage
Reagent 1	Alcohol dehydrogenase	220μl	-20℃, avoid repeated freezing and thawing.
Reagent 2	Color development solution	1.1ml	-20℃, keep away from light; avoid repeated freezing and thawing.

Reagent 3	NADH	5mg	-20℃, must be stored away from light; after NADH is prepared into solution, it must be appropriately divided and
Reagent 4	NADH preparation solution	0.8ml	-20℃, avoid repeated freezing and thawing.
Reagent 5	NAD $^+$/NADH Extract	50ml	-20℃, avoid repeated freezing and thawing.
Reagent 6	Reaction buffer	1 2 ml	-20℃, avoid repeated freezing and thawing.
Consumables 1	96-well ELISA plate	1 plate	RT
Consumables 2	96-well membrane	2 pieces	RT

Storage conditions

The unopened kit can be stored at -20℃ for 12 months.

Preparation before the experiment

Sample processing

1. Preparation of cell samples: For adherent cells, about 1×10 ^{6} cells (equivalent to the number of cells that grow in one well of a 6-well plate), aspirate the culture medium, add 200μl of ice-cold NAD $^+$/NADH extract with a pipette , and gently blow to promote cell lysis; for suspended cells, collect about 1×10 ^{6} cells, centrifuge at 600g for 5 minutes, aspirate the culture medium, add 200μl of ice-cold NAD $^+$/NADH extract with a pipette , and gently blow to promote cell lysis. Then centrifuge at 12,000g, 4ºC for 5-10 minutes, take the supernatant as the sample to be tested, and store it in an ice bath for later use.
2. Preparation of tissue samples: After washing the tissue with ice-cold PBS, weigh about 10-30 mg of tissue sample, chop it with scissors, put it in a homogenizer, add 400 μl ice-cold NAD + / NADH extraction solution , and homogenize it on ice or at room temperature. Then centrifuge at 12,000g, 4ºC for 5-10 minutes, take the supernatant as the sample to be tested, and store it in an ice bath for later use.

· Preparation of the kit

1. Preparation of NADH standard: Pipette 655μl NADH preparation solution and fully dissolve 5mg NADH provided in this kit to obtain 10mM NADH standard. Please appropriately aliquot the 10mM NADH standard and store it at -80ºC in the dark.

2. Setting up the NADH standard curve: Dilute the 10mM NADH standard with NAD $^+$/NADH extract to an appropriate concentration gradient. For example, for the initial test, you can set the concentrations to 0, 0.6 , 25 , 1.25 , 2.5 , 5 , and 10μM. When testing, add 20μl of the standard to each well of the 96-well plate, which is equivalent to 0, 12.5 , 25 , 50 , 100 , and 200pmol of NADH per well. If necessary, in subsequent experiments, the concentration range of the standard can be appropriately adjusted according to the NADH content in the sample. The point with a concentration of 0μM is the blank control point, which only contains NAD $^+$/NADH extract. Note: Since NADH is very unstable, it needs to be used as soon as possible after preparation.
3. Preparation of alcohol dehydrogenase working solution: dilute alcohol dehydrogenase 45 times with reaction buffer. For example, add 2μl of alcohol dehydrogenase to 88μl of reaction buffer to obtain 90μl of alcohol dehydrogenase working solution. Each standard or sample requires 90μl of alcohol dehydrogenase working solution. Please prepare an appropriate amount of alcohol dehydrogenase working solution according to the number of standards and samples to be tested, and make sure to prepare it before use.

Operation process

1. NAD $^+$ and NADH in the sample: Pipette 20 μl of the sample to be tested into a 96-well plate. In order to reduce experimental errors, it is recommended to set up duplicate wells for the sample. If the total amount of NAD $^+$ and NADH in the sample is found to be too high and exceeds the range of the standard curve, the sample needs to be appropriately diluted with NAD $^+$/NADH extract before testing; if the total amount is too low, the amount of cell or tissue sample needs to be increased.
2. NADH$^+$ content or NAD $^+$/NADH ratio in samples : Pipette 50-100μl of the sample to be tested into a centrifuge tube and heat it in a 60ºC water bath or PCR instrument for 30 minutes to decompose NAD $^+$. If insoluble matter is produced after heating, centrifuge at 10,000g for 5 minutes at room temperature or 4ºC, and pipette 20μl of supernatant as the sample to be tested into a 96-well plate. In order to reduce experimental errors, it is recommended to set up duplicate wells for the sample. If it is found that the NAD $^+$ or NADH content in the sample is too high and exceeds the range of the standard curve, the sample needs to be appropriately diluted with NAD $^+$/NADH extract before testing; if the content is too low, it is necessary to increase the amount of cell or tissue sample.
3. Please refer to the table below to set up blank control wells, standard wells, and sample wells in a 96-well plate. Add the alcohol dehydrogenase working solution and mix thoroughly.

	Blank control well	Standard well	Sample well

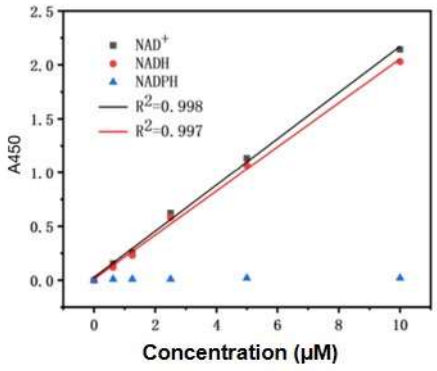
Samples to be tested	—	20 μl	20 μl
N AD $^{+}$/NADH extract	20 μl	—	—
Alcohol dehydrogenase working solution	90 μl	90 μl	90 μl

4. Incubate at 37ºC in the dark for 10 minutes. Note: The purpose of this incubation step is to convert NAD $^{+}$ in the sample into NADH; be gentle when adding the alcohol dehydrogenase working solution to avoid bubbles. If bubbles are accidentally generated, use a small pipette or needle to puncture them.
5. Mix the colorimetric solution appropriately, then add 10μl of colorimetric solution to each well, mix well, and incubate at 37ºC in the dark for 30 minutes. Orange-yellow formazan will be formed. Measure the absorbance at 450nm. If the color is lighter, the incubation time can be appropriately extended to 45-60 minutes.

Result Calculation

1. Calculate the average absorbance of each point in the standard group and subtract the absorbance of the blank control group to obtain the absorbance of each standard.
2. The standard curve is drawn with the concentration of NADH as the horizontal axis and the absorbance as the vertical axis. Please refer to the figure below for the detection effect of the NADH standard.

   The figure above shows that this kit can detect the content of NAD $^{+}$ and NADH well, and will not be interfered by NADPH. Under different detection conditions, the actual readings will vary due to the preparation of the standard, the detection instrument, etc. The data in the figure are for reference only.
3. The total concentration of NAD^{+} and NADH or the concentration of NADH in samples such as



cells and tissues is calculated based on the standard curve. Before heating at 60°C, the total concentration of NAD $^{+}$ and NADH in the sample (NAD total) is detected; after heating at 60°C, the concentration of NADH in the sample is detected. Based on the concentrations detected and the volume of the sample, the amount of NAD $^{+}$, NADH, and NAD total can be calculated.

4. According to the following calculation formula, the amount of NAD $^+$ and the ratio of NAD $^+$ /NADH in the sample are calculated. At this time, the total amount of NAD $^+$ and NADH or their respective contents can be expressed as the content per unit cell number or per unit tissue weight.
   [NAD $^+$] = [NAD total] - [NADH]
   [NAD $^+$]/[NADH] = ([NAD total] - [NADH])/[NADH]

Notes

1. All reagents in this kit need to be stored frozen. Please store them strictly according to the storage conditions. If you do not use them all at once, please store them in appropriate aliquots to avoid repeated freezing and thawing that may cause the product to become ineffective.
2. NADH is not very stable, please use it as soon as possible after taking it out . If you find that the standard curve is not ideal, it is very likely that the standard has been degraded.
3. Since the NAD $^+$ /NADH extract is relatively viscous, when using this extract as a diluent, whether diluting the standard or the sample, it is important to ensure that the dilution is uniform during the dilution process, otherwise it will easily cause large fluctuations in the experimental data.
4. During sample addition and mixing, try to avoid generating bubbles to avoid affecting the final absorbance measurement.
5. If the reaction temperature and reaction time cannot be strictly controlled, a standard curve needs to be set up for each test.
6. NAD $^+$ and NADH in the sample solution are too high or too low and are not within the linear detection range of the kit, the amount of sample or extract can be adjusted appropriately.
7. Since NAD $^+$ and NADH are very unstable and easily degraded during freezing, it is advisable to use fresh samples for testing as much as possible.
8. This product is limited to scientific research by professionals and must not be used for clinical diagnosis or treatment, used as food or medicine, or stored in ordinary residences.