Technical Manual

$NADP^+$/NADPH Colorimetric Assay Kit

- **Catalogue Code: MAES0200**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.02-5.0 μmol/L

Sensitivity:

0.02 μmol/L

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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 60oC 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 $NADP^+$/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.

5. Kit Components & Storage

Item	Specification	Storage
Extracting Solution	60 mL × 2 vials	-20°C, 6 months
Buffer Solution	12 mL × 1 vial	-20°C, 6 months
Chromogenic Agent	1.2 mL × 2 vials	-20°C, 6 months, avoid direct sunlight
Enzyme Reagent	Lyophilized × 2 vials	-20°C, 6 months
NADPH Standard	Lyophilized × 1 vial	-20°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Water bath
- 10 KD filters tube
- Microplate reader (450 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Ultrapure wate

6. Assay Notes:

1. The sample must be fresh.

2. After heat the prepared sample in a water bath at 60°C for 30 minutes, if there is turbidity, centrifuge at 10000 g at 4°C for 10 minutes and then take the supernatant for detection.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **Enzyme working solution:** Dissolve a vial of enzyme reagent 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°C for 7 days and avoid direct sunlight.
   Preparation of **reaction working solution:** Mix the enzyme working solution and buffer solution at the ratio of 1: 49 fully. Prepare the fresh solution before use and store avoiding direct sunlight.

3. Preparation of **NAPDH Standard (1 mmol/L):** Dissolve a vial of NADPH standard 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°C for 7 days and avoid direct sunlight.

4. Preparation of **NAPDH Standard solution (10 μmol/L):** Dilute **1 mmol/L standard** with extracting solution at the ratio of 1:99. Prepare the fresh solution before use

8. Sample Preparation

1. Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of extracting solution (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

2. 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 (4×10^6): extracting solution (μL) =1: 800. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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 extracting solution.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 450 nm

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.

10. Operation Steps

The preparation of standard curve

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.

The preparation of standard curve

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°C 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°C 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°C for 10 min. Measure the OD value of each well at 450 nm with microplate reader.

Operation Table

	Standard well	Sample well
Sample supernatant (μL)		50
Standard of different concentrations (μL)	50	
Reaction working solution (μL)	100	100
Mix fully with microplate reader for 5 s and incubate at 37°C for 10 min.		
Chromogenic agent (μL)	20	20
Mix fully with microplate reader for 5 s and incubate at 37°C for 10 min. Measure the OD value of each well at 450 nm with microplate reader.		

11. Calculations

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:

$$\frac{NADP_{total}}{(\mu mol/gprot)} = \frac{\Delta A_1 - b}{a} \times f \div C_{pr}$$

2. For NADPH:

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

3. For $NADP^+$:

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

4. For $NADP^+$/NADPH:

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

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_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, mgprot/mL

12. Performance Characteristics

Detection Range	0.02-5.0 μmol/L
Sensitivity	0.02 μmol/L
Average inter-assay CV (%)	5.5
Average intra-assay CV (%)	2.1

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:

$$\underset{(\mu mol/gprot)}{NADP_{total}} = (0.654 - 0.075 + 0.0232) \div 0.3807 \div 0.063$$

$$= 25.11\ \mu mol/gprot$$

$$\underset{(\mu mol/gprot)}{NADPH} = (0.400 - 0.075 + 0.0232) \div 0.3807 \div 0.063$$

$$= 14.52\ \mu mol/gprot$$

$$\underset{(\mu mol/gprot)}{NADP^+} = 25.11 - 14.52$$

$$= 10.59\ \mu mol/gprot$$

$$\frac{NADP^+}{NADPH} = 10.59 \div 25.11 \times 100\%$$

$$= 42.3\%$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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Notes:

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