TECHNICAL MANUAL

NADP+/NADPH Colorimetric Assay Kit

- **SKU CODE:** MAES0200
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Sample pretreatment
- Add standards and samples
- Incubation and measurement

2. Intended use

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

3. Detection principle

Detection of 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, transfers electrons to WST-8 to produce a yellow product, which has a characteristic absorption peak at 450 nm. Therefore, the total content of NADP+ and NADPH can be quantified by measuring the OD value at 450 nm.

Detection of NADPH:

After treating the sample and heating at 60°C water bath for 30 min, the NADP+ in the sample is decomposed and only NADPH remains. NADPH reduces WST-8 to form formazan, and the amount of NADPH is determined by measuring the OD value at 450 nm.

Detection of NADP+ and NADP+/NADPH ratio:

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

Note: NAD+ and NADH have no effect on the determination results.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	-20°C, 12 months
Reagent 2	Buffer Solution	12 mL × 1 vial	-20°C, 12 months
Reagent 3	Chromogenic Agent	1.2 mL × 2 vials	-20°C, 12 months, protect from light
Reagent 4	Enzyme Reagent	Powder × 2 vials	-20°C, 12 months
Reagent 5	NADPH Standard	Powder × 1 vial	-20°C, 12 months, protect from light
	Microplate	96 wells	
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Micropipettor, 37°C water bath, Microplate reader (450 nm), 10 kD filter tubes

Reagents:

Ultrapure water, PBS (0.01 M, pH 7.4)

6. 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 immediately before use. Store at -20°C for up to 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 immediately before use and stored protected from light.
4. **Preparation of 1 mmol/L NADPH Standard:** Dissolve one vial of NADPH standard with 4.8 mL of ultrapure water, mix well to dissolve. The 1 mmol/L NADPH standard should be prepared immediately before use. Aliquot and store at -20°C for up to 7 days, protected from light.

5. **Preparation of 10 μmol/L NADPH standard solution:** Before testing, prepare sufficient 10 μmol/L NADPH standard solution according to the test wells. For example, prepare 1000 μL of 10 μmol/L NADPH standard solution (mix well 10 μL of 1 mmol/L NADPH standard and 990 μL of extracting solution). The 10 μmol/L NADPH standard solution should be prepared immediately before use.
6. **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 serial concentrations. The recommended dilution gradient is as follows: 0, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 μmol/L.

7. 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°C.

4. Centrifuge at 10,000 × g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

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. Pipette gently and allow to stand for 10 min to lyse cells.

4. Centrifuge at 12,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only): Jurkat cells (1), HEK293T 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 perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The sample must be fresh.
2. After heating the prepared sample in a water bath at 60°C for 30 minutes, if there is turbidity, centrifuge at 10,000×g at 4°C for 10 minutes and then take the supernatant for detection.

9. Operating steps

Sample pretreatment:

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

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

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. Add 100 μL of reaction working solution into each well.

3. Mix thoroughly 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 thoroughly 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.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and corresponding 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 (μmol/g_{prot}) = (ΔA_1 - b) ÷ a × f ÷ C_{pr}

2. For NADPH:

[NADPH] (μmol/g_{prot}) = (ΔA_2 - b) ÷ a × f ÷ C_{pr}

3. For NADP+:

[NADP+] (μmol/g_{prot}) = [NADP]_total - [NADPH]

4. For NADP+/NADPH:

[NADP+] / [NADPH] = ([NADP]_total - [NADPH])/ [NADPH] ×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, g_{prot}/L.

11. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

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

Inter-assay Precision

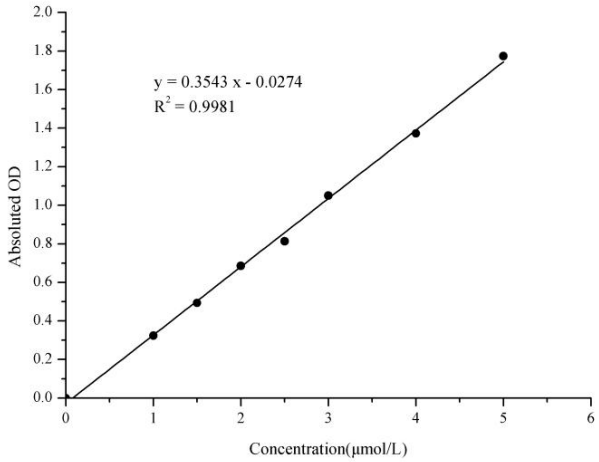
Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

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.

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), the standard curve and data are provided below for reference only:



12. Appendix II Example Analysis

Example analysis:

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

Standard curve: y = 0.3807x – 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 g_{prot}/L, and the calculation result is:

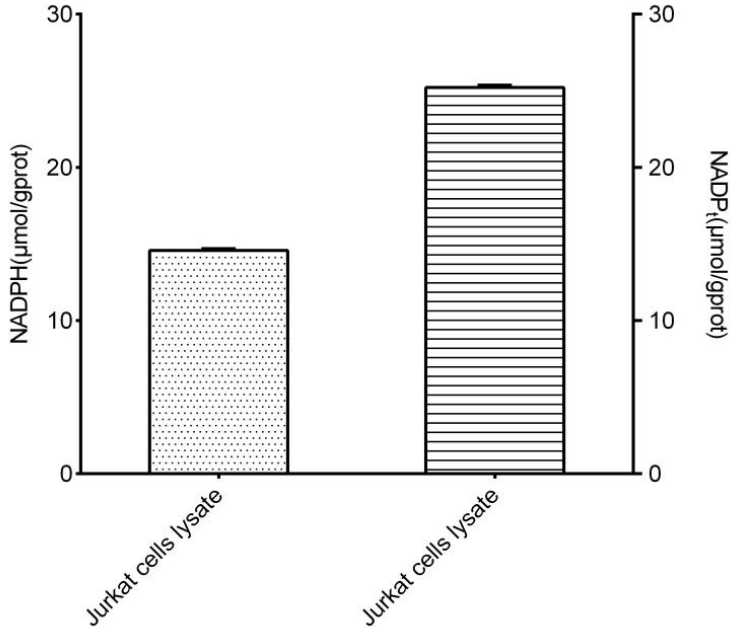
[NADP]_total (µmol/g_{prot}) = (0.654 - 0.075 + 0.0232) ÷ 0.3807 ÷ 0.063 = 25.11 µmol/g_{prot}

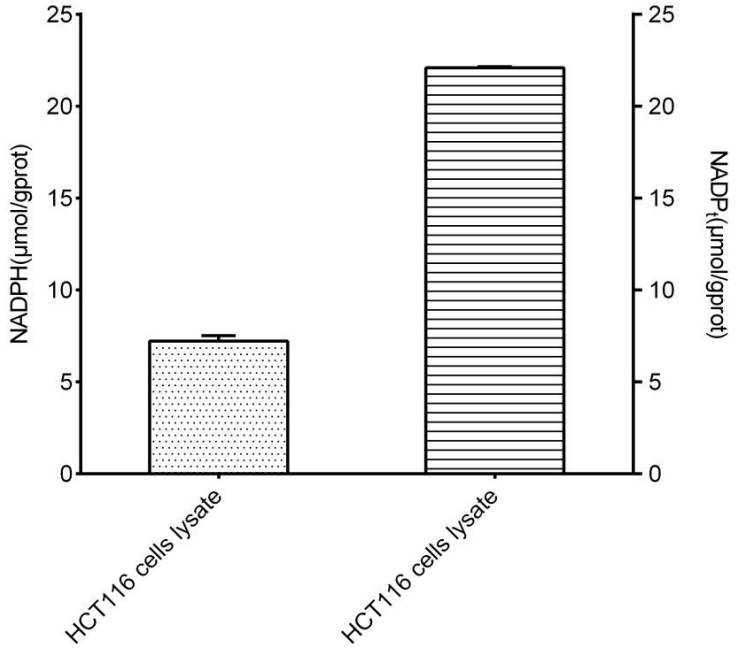
[NADPH] (µmol/g_{prot}) = (0.400-0.075 + 0.0232) ÷ 0.3807 ÷ 0.063 = 14.52 µmol/g_{prot}

[NADP+] (µmol/g_{prot}) = 25.11- 14.52 = 10.59 µmol/g_{prot}

[NADP+] / [NADPH] = 10.59 ÷ 14.52 × 100% = 72.9%

Detected Jurkat cells (the concentration of protein is 0.063 g_{prot}/L) and HCT116 cells (the concentration of protein is 0.068 g_{prot}/L) according to the protocol, the result is as follows:





13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.