

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

Catalog No: E-BC-F170

Specification: 48T(32 samples)/96T(80 samples)

Measuring instrument: Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range: 8.55-1000 nmol/L

Elabscience® NADPH Fluorometric Assay Kit

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

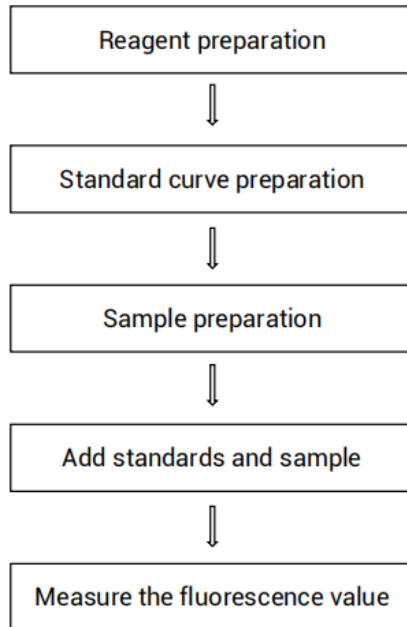
Website: www.elabscience.com

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

The kit is suitable for detecting the content of NADPH in animal tissues and cells.

Detection principle

Glucose-6-phosphate (G6P) is oxidized by glucose-6-phosphate dehydrogenase (G6PDH) to gluconolactone-6-phosphate (6-PG). At the same time, NADP⁺ is reduced to NADPH in this reaction. The generated NADPH reacts with a chromogenic agent under enzymatic catalysis to produce a fluorescent substance. The fluorescence intensity can be measured to calculate the NADPH content in the sample.

Kit components & storage

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|-----------|---------------------|-----------------|------------------|---------------------------------|
| Reagent 1 | Extract Solution | 46 mL × 1 vial | 46 mL × 2 vials | -20°C, 12 months |
| Reagent 2 | Buffer Solution | 6 mL × 1 vial | 12 mL × 1 vial | -20°C, 12 months |
| Reagent 3 | Enzyme Reagent | Power × 1 vial | Power × 2 vials | -20°C, 12 months, shading light |
| Reagent 4 | Substrate | 1.1 mL × 1 vial | 1.1 mL × 2 vials | -20°C, 12 months |
| Reagent 5 | Chromogenic Agent | 0.1 mL × 1 vial | 0.2 mL × 1 vial | -20°C, 12 months, shading light |
| Reagent 6 | Standard | Power × 1 vial | Power × 2 vials | -20°C, 12 months, shading light |
| | Black Microplate | 96 wells | | No requirement |
| | Plate Sealer | 2 pieces | | |
| | Sample Layout Sheet | 1 piece | | |

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator, Water bath

Materials required but not provided

Distilled or deionized water, PBS (0.01 M, pH 7.4), 10 kDa MWCO Spin Filter (Outer tube 1.5 mL, Inter tube 0.5 mL)

Reagent preparation

- ① Equilibrate all the reagents to 25°C before use.
- ② Enzyme Working Solution preparation:
Dissolve one vial of Enzyme Reagent with 275 μL of distilled or deionized water. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.
- ③ Working Solution preparation:
Before testing, please prepare sufficient Working Solution. For example, prepare 626 μL of Working Solution (mix well 500 μL of Buffer Solution, 25 μL of Enzyme Working Solution, 100 μL of Substrate and 1 μL of Chromogenic Agent). The Working Solution should be freshly prepared before use. Stable for 4 h protected from light.

④ 1000 nmol/L Standard Solution preparation:

Dissolve one vial of Standard with 200 μL of distilled or deionized water to prepare a 10 mmol/L Standard Solution. The unused 10 mmol/L Standard Solution can be stored at -20°C for 7 days protected from light. Mix well 990 μL of Extract Solution and 10 μL of 10 mmol/L Standard Solution to prepare a 100 $\mu\text{mol/L}$ Standard Solution, which is valid for 8 hours when protected from light. Mix well 990 μL of Extract Solution and 10 μL of 100 $\mu\text{mol/L}$ Standard Solution to prepare a 1000 nmol/L Standard Solution. Stable for 8 h protected from light.

⑤ Standard curve preparation:

Always prepare a fresh set of standards. Discard Working Standard Dilutions after use.

Dilute 1000 nmol/L Standard Solution with Extract Solution to a serial concentration. The recommended dilution gradient is as follows: 0, 50, 100, 200, 400, 600, 800, 1000 nmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|--|----------|-----------|------------|------------|------------|------------|------------|-------------|
| Concentration (nmol/L) | 0 | 50 | 100 | 200 | 400 | 600 | 800 | 1000 |
| 1000 nmol/L Standard (μL) | 0 | 10 | 20 | 40 | 80 | 120 | 160 | 200 |
| Extract Solution (μL) | 200 | 190 | 180 | 160 | 120 | 80 | 40 | 0 |

Sample preparation

Tissue sample:

- ① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).
- ② Wash tissue in cold PBS (0.01 M, pH 7.4).
- ③ Homogenize 50 mg tissue in 450 μ L extract solution with a dounce homogenizer at 4°C.
- ④ Centrifuge at 10000 \times g for 10 min at 4°C to remove insoluble material. Transfer the supernatant to 10kDa MWCO Spin Filter. Centrifuge at 12000 \times g for 25 min. Collect the outer tube filtrate, heat it in a water bath at 60°C for 30 min, cool it under running water, and preserve it on ice for detection.

Cell sample

- ① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
- ② Wash cells with PBS (0.01 M, pH 7.4).
- ③ Lyse 1×10^6 cells with 200 μ L extraction solution. Place on the ice box and mix well every 5 min, lyse for 10 min.
- ④ Centrifuge at 10000 \times g for 10 min at 4°C to remove insoluble material. Transfer the supernatant to 10kDa MWCO Spin Filter. Centrifuge at 12000 \times g for 25 min. Collect the outer tube filtrate, heat it in a water bath at 60°C for 30 min, cool it under running water, and preserve it on ice for detection.

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

| Sample type | Dilution factor |
|---------------------------------------|-----------------|
| 10% Mouse liver tissue homogenization | 1 |
| 10% Mouse heart tissue homogenization | 1 |
| 1×10^6 HL 60 cells | 1 |
| 1×10^6 RAW264.7 cells | 1 |
| 1×10^6 A549 cells | 1 |
| 1×10^6 K562 cells | 1 |

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

- ① Standard well: Add 20 μ L of Standard Solution with different concentrations to the corresponding wells.
Sample well: Add 20 μ L of sample to the corresponding wells.
- ② Add 120 μ L of working solution into each well.
- ③ Shake the microplate for 5 seconds to ensure complete mixing. Incubate at 37°C for 10 min. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the corrected fluorescence value.
3. Plot the standard curve by using corrected fluorescence 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. Tissue sample:

$$\text{NADPH content (nmol/kg wet weight)} = (\Delta F - b) \div a \times V \div m \times f$$

3. Cell sample:

$$\text{NADPH content (nmol/10}^9) = (\Delta F - b) \div a \times V \div n \times f \times 1000$$

[Note]

$$\Delta F: \Delta F = F_{\text{sample}} - F_{\text{blank}}$$

m: The weight of tissue, kg.

V: The volume of extract solution, L.

n: The number of cell sample/ 10^6 .

f: Dilution factor of sample before tested.

1000: Conversion factor.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three A549 cell 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 (nmol/L) | 470 | 500 | 468 |
| %CV | 5.0 | 1.7 | 2.3 |

Inter-assay Precision

Three A549 cell samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---------------|----------|----------|----------|
| Mean (nmol/L) | 470 | 500 | 468 |
| %CV | 8.4 | 4.3 | 3.3 |

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99.7%.

| | Sample 1 | Sample 2 | Sample 3 |
|-------------------------|----------|----------|----------|
| Expected Conc. (nmol/L) | 470 | 500 | 468 |
| Observed Conc. (nmol/L) | 488.8 | 485 | 458.6 |
| Recovery rate (%) | 104 | 97 | 98 |

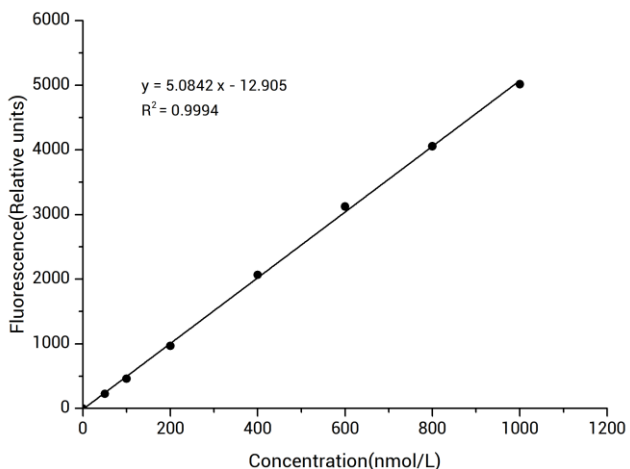
Sensitivity

The analytical sensitivity of the assay is 8.55 nmol/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 fluorescence 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 (nmol/L) | 0 | 50 | 100 | 200 | 400 | 600 | 800 | 1000 |
|------------------------------|-----|------|------|------|------|------|------|------|
| Fluorescence value | 798 | 1029 | 1250 | 1760 | 2827 | 3897 | 4844 | 5698 |
| | 788 | 1011 | 1260 | 1758 | 2891 | 3935 | 4850 | 5916 |
| Average fluorescence value | 793 | 1020 | 1255 | 1759 | 2859 | 3916 | 4847 | 5807 |
| Corrected fluorescence value | 0 | 227 | 462 | 966 | 2066 | 3123 | 4054 | 5014 |



Appendix Π Example Analysis

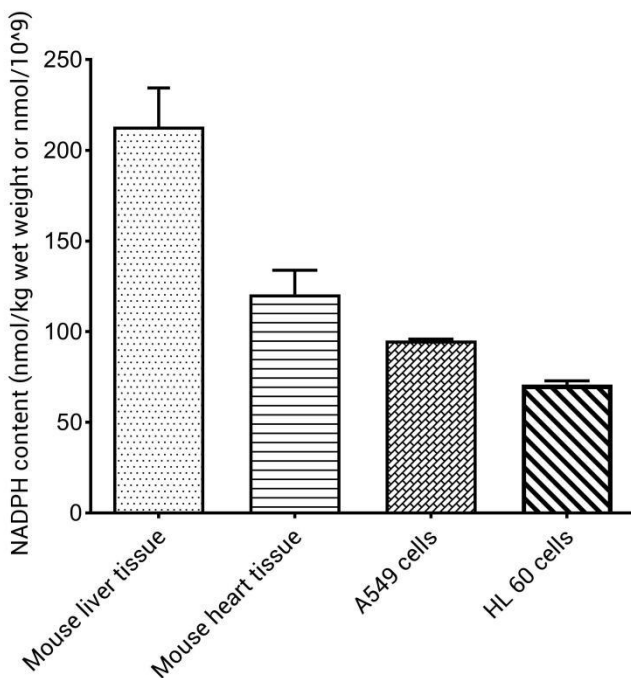
Example analysis:

Take 20 μL of A549 cell filtrate to the well of microplate. Proceed according to the operating steps. The results are as follows:

standard curve: $y = 5.0842x - 12.905$, the average fluorescence value of the blank well is 793, the average fluorescence value of the sample well is 3198, $\Delta F = F_{\text{sample}} - F_{\text{blank}} = 3198 - 793 = 2405$, and the calculation result is:

$$\begin{aligned}\text{NADPH content (nmol}/10^9) &= (2405 + 12.905) \div 5.0842 \times 0.0002 \div 1 \times 1000 \\ &= 95.11 \text{ nmol}/10^9\end{aligned}$$

Detect 10% mouse liver tissue homogenate supernatant, 10% mouse heart tissue homogenate supernatant, 1×10^6 A549 cells and 1×10^6 HL 60 cells according to the protocol, the result is as follows:



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.

