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

Catalog No：E-BC-F025

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=510 nm/560 nm)

Elabscience® N-Acetylcysteine (NAC) 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
Tel: 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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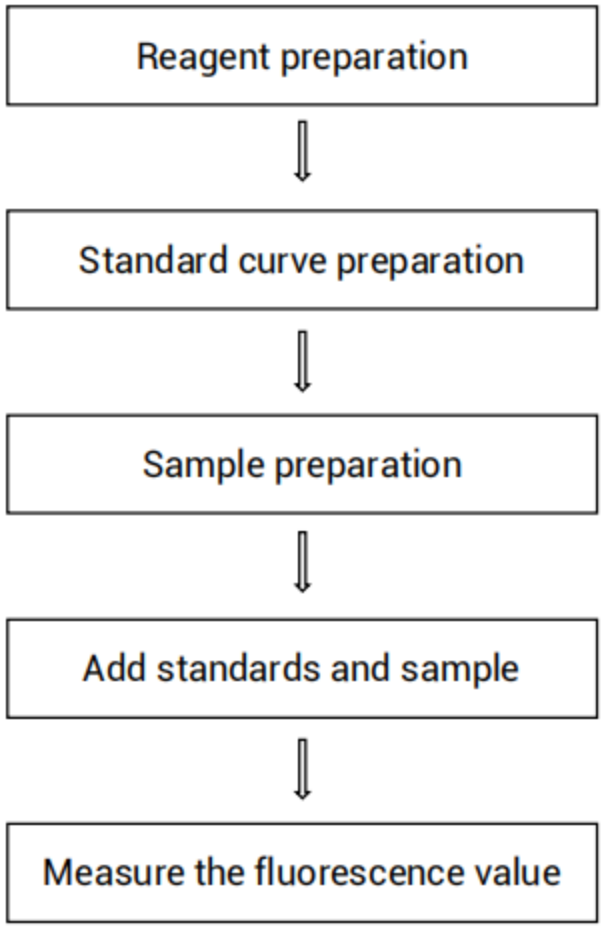
Assay summary



Intended use

This kit is used for the determination of the N-acetylcysteine (NAC) content in serum, plasma, tissue, and cell samples.

Detection principle

N-Acetylcysteine (NAC) is a precursor for the biosynthesis of cysteine and glutathione. It is an antioxidant and free radical scavenger, commonly used in the treatment of various diseases.

The principle of this kit is as follows: the NAC probe reacts with NAC to generate fluorescence, which can be measured at excitation and emission wavelengths of 510 nm and 560 nm, respectively. The NAC content in samples is then quantified by comparing the fluorescence values with those of NAC standards.

Kit components & storage

Item	Component	Size1 (48 T)	Size2 (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	50 mL × 2 vials	-20℃,12 months
Reagent 2	Substrate	0.5 mL × 1 vial	1 mL × 1 vial	-20℃, 12 months shading light
Reagent 3	Diluent	Powder × 2 vials	Powder × 4 vials	-20℃, 12 months
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Fluorescence microplate reader (Ex/Em=510 nm/560 nm), Incubator (37℃), Vortex mixer

Reagent ：

Double and distilled water

Instruments

Fluorescence microplate reader (Ex/Em=510 nm/560 nm), Incubator (37℃), Vortex mixer

Materials required but not provided

Double and distilled water, 10kDa MWCO Spin Filter (Inner tube 0.5 mL, outer tube 1.5 mL)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of working solution：

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 200 μL of working solution (mix well 198 μL of buffer solution and 2 μL of probe). Keep it on ice during use protected from light and used up within 4 hours.

③ The preparation of 1 mmol/L standard:

One vial of Reagent 3 should be dissolved and brought to a final volume of 50 mL with double distilled water. The resulting solution should be kept on ice, protected from light, and used immediately after preparation. Keep it on ice during use protected from light and used up within 4 hours.

④ The preparation of 50 μmol/L standard:

Before testing, please prepare sufficient 50 μmol/L standard according to the test wells. For example, prepare 100 μL of working solution (mix well 95 μL of double and distilled water and 5 μL of 1 mmol/L standard). Keep it on ice during use protected from light and used up within 2 hours.

⑤ The preparation of 1μmol/L standard:

Before testing, please prepare sufficient 1μmol/L standard according to the test wells. For example, prepare 1000 μL of 1μmol/L standard (mix well 980 μL of distilled water and 20 μL of 50 μmol/L standard). Keep it on ice during use protected from light and used up within 2 hours.

⑥ The preparation of standard curve:

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

Dilute 1 μmol/L standard solution with double and distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8, 1.0 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.7**	**0.8**	**1.0**
1 μmol/L Standard (μL)	0	40	60	80	120	140	160	200
Double and distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

Serum or plasma samples: Collect 200-400 µL samples and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

Tissue sample:

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

② Homogenize 20 mg tissue in 180 µL double and distilled water with a dounce homogenizer at 4℃.

③ Centrifuge at 12000×g for 10 min at 4℃ to remove insoluble material.

④ Collect 200-400 µL supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑤ Take the filtered sample supernatant and preserve it on ice for detection. The supernatant should be used up within 4 h (Samples need to be stored at 4℃, as temperature can affect the test results).

Cell (adherent or suspension) samples:

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

② Homogenize 1×10^6 cells in 200 µL double and distilled water with a ultrasonic cell disruptor at 4℃.

⑥ Centrifuge at 12000×g for 10 min at 4℃ to remove insoluble material.

⑦ Collect 200-400 µL supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑧ Take the filtered sample supernatant and preserve it on ice for detection. The supernatant should be used up within 4 h (Samples need to be stored at 4℃, as temperature can affect the test results).

Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	80-100
10% Mouse kidney tissue homogenate	80-100
1×10^6 RAW 264.7 cells	20-30
1×10^6 HepG2 cells	20-30
Human serum	2-5
Mouse serum	2-5
Rat serum	2-5
Rabbit serum	2-5

Note: The diluent is double and distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 20 μL of standard solution with different concentrations into the wells.

Sample well: add 20 μL of sample into the wells.

② Add 150 μL of working solution into each well.

③ Mix fully with fluorescence microplate reader for 3 s and incubate at 37℃ for 10 min.

④Measure the fluorescence intensity of each well at the excitation wavelength of 510 nm and the emission wavelength of 560 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 absoluted fluorescence value.

3. Plot the standard curve by using absoluted 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. serum and plasma samples:

$$\text{NAC content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue samples:

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

2. Cell sample:

$$\text{NAC content (μmol/10^6)} = (\Delta F - b) \div a \times f \div \frac{n}{V}$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{control}$.

m: The weight of tissue, g.

V: The volume of extraction working solution, 0.02 mL.

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

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse serum 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)	1.00	3.25	4.32
%CV	1.3	1.0	2.5

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.00	3.25	4.32
%CV	5.9	6.7	8.2

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 99.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc(μmol/L)	1.00	3.25	4.32
Observed Conc(μmol/L)	0.91	3.08	24.95
Recovery rate (%)	91.0	95	4.15

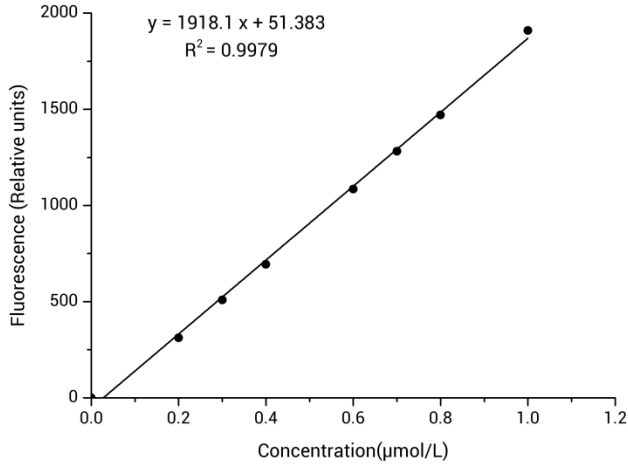
Sensitivity

The analytical sensitivity of the assay is 0.057 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 fluorescence 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	0.2	0.3	0.4	0.6	0.7	0.8	1.0
F value	466	788	980	1155	1543	1726	1928	2390
	492	794	996	1190	1585	1796	1970	2388
Average F value	479	791	988	1173	1564	1761	1949	2389
Corrected F value	0	312	509	694	1085	1282	1470	1910



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.