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

Catalog No：E-BC-F048

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.89-100 μmol/L

Elabscience® γ-Aminobutyric Acid (GABA) 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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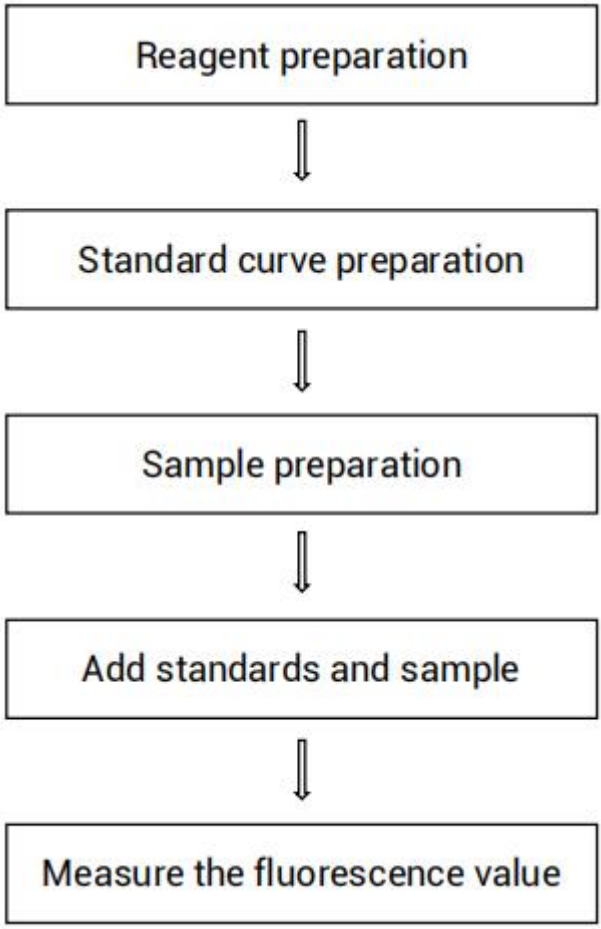
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Assay summary



Intended use

This kit can be used for determination of γ-Aminobutyric Acid (GABA) content in serum, plasma, tissue and cell samples.

Detection principle

γ-Aminobutyric Acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system. GABA also has the function of promoting mental stability, regulating blood pressure level, improving brain vitality, increasing growth hormone secretion, accelerating ethanol metabolism and providing nutrients to nerve cells.

GABA is catalyzed by enzyme, and the removed hydrogen will reduce $NADP^+$ to NADPH, and the GABA content of the sample can be calculated by measuring the generated amount of NADPH.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Matrix Solution	12 mL × 1 vial	24 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Accelerant	0.24 mL × 1 vial	0.48 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Substrate	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months shading light
Reagent 4	Enzyme Reagent	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months shading light
Reagent 5	1 mmol/L Standard Solution	1 mL × 1 vial	1 mL × 2 vials	-20°C, 12 months shading light
	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=535 nm/587 nm), Incubator (37℃)

Reagents:

Normal saline (0.9% NaCl)

Consumptive material:

3kDa MWCO Spin Filter

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of Working Solution：

a. Solution A: Take 5.88 mL Matrix Solution, 120 μL Accelerrant, mix well.

b. Working solution: Dissolve one vial of Substrate and one vial of Enzyme Reagent with 6 mL of Solution A, mix well to dissolve. The Working Solution should be prepared on spot and used up within 1 day.

③ The preparation of 100 μmol/L Standard Solution：

Dilute 100 μL of 1 mmol/L Standard Solution with 900 μL of double distilled water, mix well. Store at 2-8°C for a week.

④ The preparation of standard curve：

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

Dilute 100 μmol/L Standard Solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 40, 50, 60, 70, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**40**	**50**	**60**	**70**	**80**	**100**
100 μmol/L Standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: Put sample into 3kDa MWCO Spin Filter and centrifuge at 12000×g for 15 min. Collect the filtrate and preserve it on ice for detection.

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 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

⑤ Collect supernatant and add it to 3kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑥ Take the filtered sample supernatant and preserve it on ice for detection.

Cell (adherent or suspension) samples:

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

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

⑤ Collect supernatant and add it to 3kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑥ Take the filtered sample supernatant and preserve it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Mouse serum	1
Rat serum	1
Porcine serum	1
10% Mouse brain tissue homogenate	3-6
10% Mouse kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse muscle tissue homogenate	1
1×10^6 RAW264.7 cells	1
1×10^6 HepG2 cells	1

Note: The diluent is normal saline (0.9% NaCl). 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 with different concentrations into the corresponding wells.
Sample well: add 20 μL of sample into the corresponding wells.

② Add 200 μL of Working Solution into each well.

③ Mix fully for 3 s with fluorescence microplate reader, Incubate at 37°C for 30 min protected from light. 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 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:

Serum and plasma samples:

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

Tissue sample:

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

Cell sample:

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

[Note]

ΔF: The absolute fluorescence value of sample, $F_{sample} - F_{blank}$ (F_{blank} is the fluorescence value when the standard concentration is 0).

m: The weight of tissue, g.

V: The volume of normal saline in the preparation step of sample, 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 rat serum 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 (μmol/L)	32.00	48.00	64.00
%CV	3.2	4.5	3.7

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	32.00	48.00	64.00
%CV	3.1	9.5	6.9

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 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	30	55	75
Observed Conc.(μmol/L)	30.9	57.2	81.0
Recovery rate (%)	103	104	108

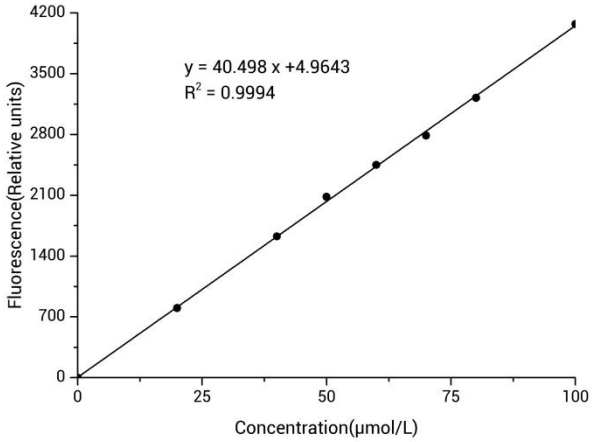
Sensitivity

The analytical sensitivity of the assay is 0.89 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	20	40	50	60	70	80	100
Fluorescence value	683	1507	2396	2751	3118	3560	3905	4769
	695	1475	2239	2792	3163	3394	3920	4755
Average fluorescence value	689	1491	2318	2772	3141	3477	3913	4762
Corrected fluorescence value	0	802	1629	2083	2452	2788	3224	4073



Appendix Π Example Analysis

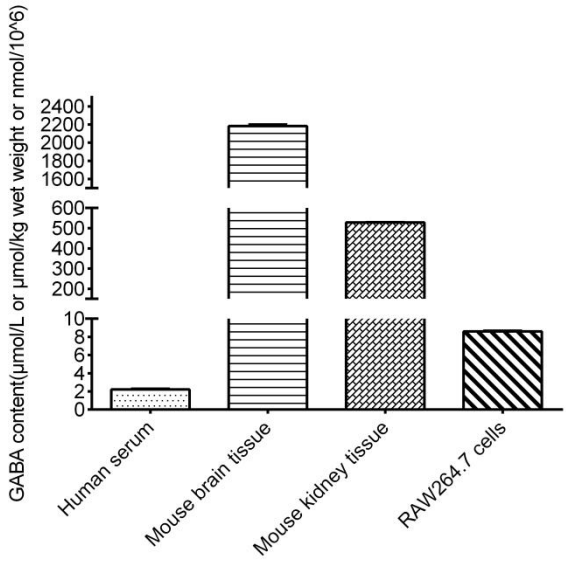
Example analysis：

Take 20 μL of 10% mouse brain tissue homogenate which dilute for 3 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 40.498x + 4.9643, the average fluorescence value of the sample is 3969, the average fluorescence value of the blank is 689, the calculation result is:

GABA concent (μmol/kg wet weight) = (3969 – 689 – 4.9643) ÷ 40.498 × 0.9 ÷ 0.1 × 3 = 2183.46 μmol/kg wet weight

Detect human serum, 10% mouse brain tissue homogenate (dilute for 3 times), 10% mouse kidney tissue homogenate and 1×10^6 RAW264.7 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.