Product Information & Manual

GABA Assay Kit (Fluorometric)

NBP3-48889

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Novus kits are guaranteed for 6 months from date of receipt.

For research use only. Not for diagnostic or therapeutic procedures.

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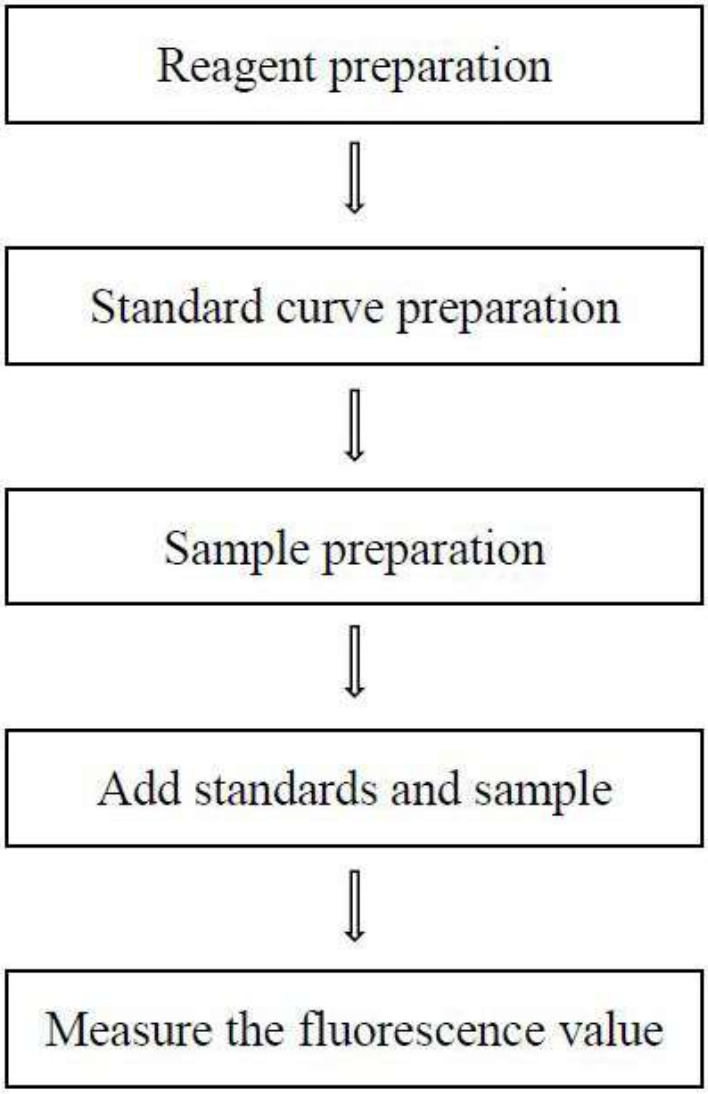
Assay summary



Intended use

This kit can be used for determination of 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	Buffer Solution	13 mL × 1 vial	26 mL × 1 vial	2-8°C, 12 months shading light
Reagent 2	Accelerrant	0.27 mL × 1 vial	0.54 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Chromogenic Agent	0.1 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Substrate	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months shading light
Reagent 5	1 mmol/L Standard Solution	1.2 mL × 1 vial	1.2 mL × 2 vials	-20°C, 12 months shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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°C)

Reagents:

Normal saline (0.9% NaCl)

Consumptive material:

3kDa MWCO Spin Filter

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of working solution：

a. Solution A: Take 5.85 mL buffer solution, 120 μL accelerrant and 30 μL chromogenic agent, mix well.

b. Working solution: Dissolve one vial of substrate 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.

□ Dilution of sample

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

Sample type	Dilution factor
Rabbit serum	15-25
Rat serum	20-40
Mouse plasma	15-25
Human serum	15-25
10% Mouse liver tissue homogenate	15-20
10% Mouse brain tissue homogenate	30-45
1×10^6 Molt-4 cells	1
1×10^6 RAW cell	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

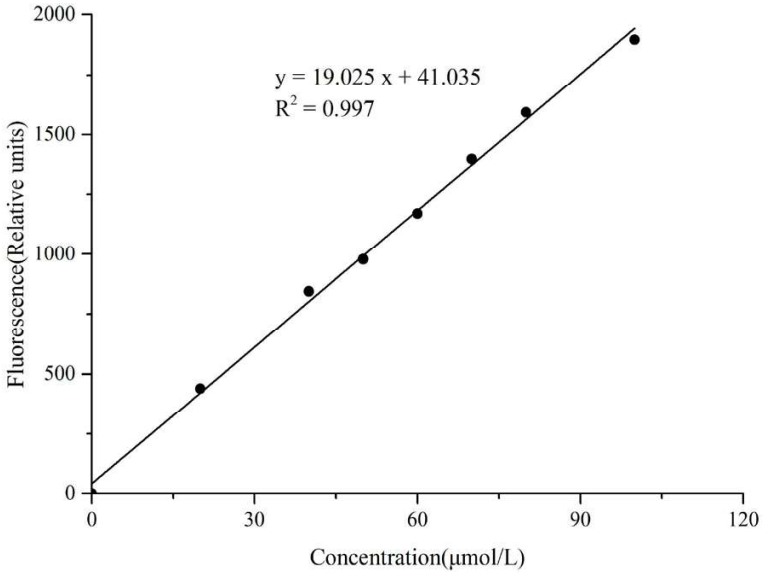
Sensitivity

The analytical sensitivity of the assay is 10.74 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	1084	1524	1900	2006	2226	2447	2623	2942
	1048	1487	1920	2082	2246	2482	2695	2983
Average fluorescence value	1066	1506	1910	2044	2236	2464	2659	2962
Absoluted fluorescence value	0	440	844	978	1170	1398	1593	1896



Appendix Π Example Analysis

Example analysis□

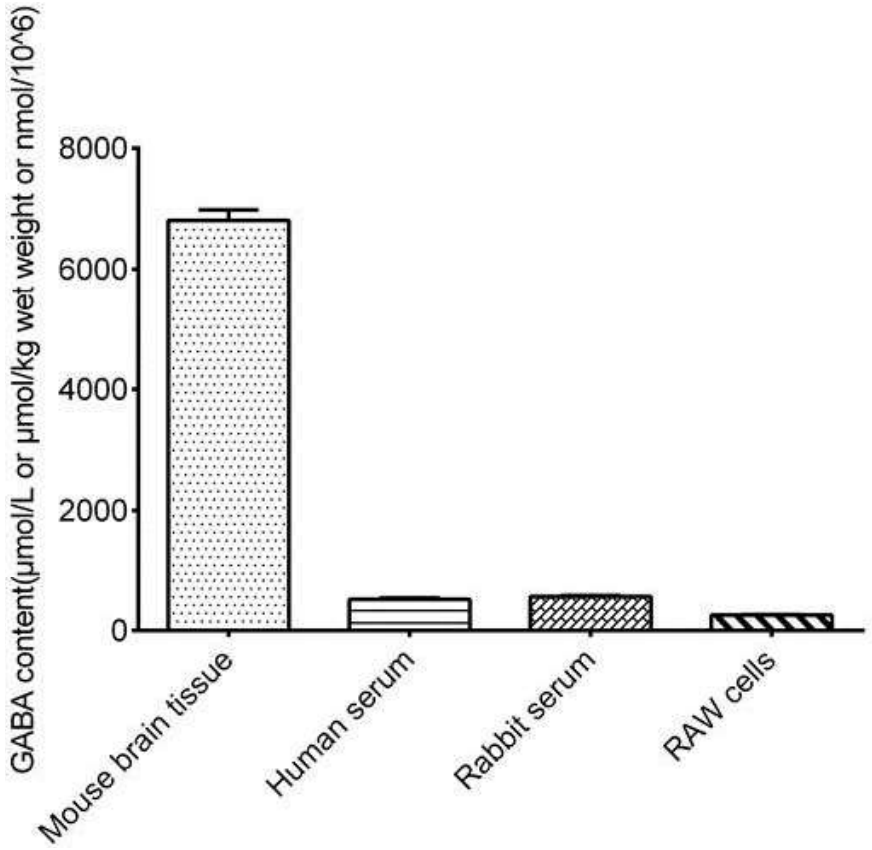
Take 20 μL of 10% mouse brain tissue homogenate which dilute for 40 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 19.025x + 41.035, the average fluorescence value of the sample is 1475, the average fluorescence value of the blank is 1066, the calculation result is:

GABA concent (μmol/kg wet weight) = (1475 – 1066 – 41.035) ÷ 19.025 × 0.9 ÷ 0.1 × 40

= 6963 μmol/kg wet weight

Detect 10% mouse brain tissue homogenate (dilute for 40 times), human serum (dilute for 20 times), rabbit serum (dilute for 20 times) and 1×10^6 RAW cells (dilute for 2 times) 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. Novus Biologicals 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.