PRODUCT INFORMATION & MANUAL

Glutamine Synthetase Activity Assay Kit (Colorimetric)

NBP3-24495

For research use only.

Not for diagnostic or therapeutic procedures.

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Glutamine Synthetase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24495

Method: Colorimetric method

Specification: 96T (Can detect 96 samples without duplication)

Instrument: Microplate reader

Sensitivity: 8.84 U/L

Detection range: 8.84–321.05 U/L

Average intra-assay CV (%): 2.5

Average inter-assay CV (%): 5.8

Average recovery rate (%): 104

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure glutamate synthase (GOGAT) activity in serum, animal and plant tissue samples.

▲Detection principle

Glutamate synthase (GOGAT) mainly exists in prokaryotes, saccharomyces yeasts and proplasts of non-green tissues of higher plants. GOGAT and glutamine synthetase (GS) constitute the GS/GOGAT cycle and participate in the regulation of ammonia assimilation.

GOGAT catalysis the reaction that transfer the amino from glutamine to a-KG to form two molecules of glutamic acid using NADH as the electron donor. The decreasing rate of NADH that can be measured at 340 nm can reflect the activity of GOGAT.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution	50 mL × 2 vials	-20℃, 12 months
Reagent 2	Buffer Solution	26 mL × 1 vial	-20°C, 12 months,
Reagent 3	Substrate A	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 4	Substrate B	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent	Powder × 2 vials	-20°C, 12 months, shading light
	UV 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.			

▲Materials prepared by users

Instruments

Microplate reader (330- 350nm, optimum wavelength: 340 nm)

⚠Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. It should be ensured that the powder in the prepared reaction working solution is completely dissolved.
2. It's better to measure no more than 4 samples at same time.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reaction working solution:

   Dissolve a vial of reagent 3, reagent 4 and reagent 5 powder with 12.5 mL of reagent 2. Prepare the needed fresh solution before use and the prepared solution can be store at 2-8°C with shading light for 12 h.

▲ Sample preparation

1. Serum and plasma samples:

   Detect directly (If the sample is turbid, centrifuge at 12000 g for 10 min before detection).

2. Tissue sample:

   Weigh 0.1g of tissue sample, add 0.9 mL of reagent 1 , homogenize the tissue in ice bath, centrifuge at 10000 g for 10 min at 4°C, then take the supernatant for measurement. The supernatant after centrifugation must be clarified, and if there is turbidity, it must be centrifuged again. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (8.84–321.05 U/L).

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

Sample type	Dilution factor
10% Rat kidney tissue homogenate	2-4
10% Rat liver tissue homogenate	2-4
10% Rat heart tissue homogenate	2-4
10% Mouse liver tissue homogenate	2-4
Bovine serum	1
10% pleurotus cornucopiae tissue homogenate	1
10% beech mushroom tissue homogenate	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96

Note:S1-S96, sample wells.

▲Detailed operation steps

(1) Sample well: Add 20 μL of sample to the wells.

(2) Add 200 μL of reaction working solution into each well.

3) Mix fully with microplate reader for 5 s. Measure the OD value of each well at 0 min recorded as A_1. Incubate at 25°C for 4 min and measure the OD value of each well recorded as A_2.

▲Summary operation table

	Sample well
Sample (μL)	20
Reaction working solution (μL)	200
Mix fully and measure the OD value of each well at 0 min recorded as A_1. Incubate at 25°C for 4 min and measure the OD value of each well recorded as A_2.	

▲Calculation

1. Serum/plasma samples:

Definition: The amount of GOGAT in 1 L serum or plasma that catalyze and decompose 1 μmol NADH in 1 minute at 25°C is defined as 1 unit.

$$\text{GOGAT activity (U/L)} = \Delta A_{340} \div (\varepsilon \times d) \times V_{total} \div V_{sample} \div T \times f \times 10^6$$

2. Tissue and cell samples:

Definition: The amount of GOGAT in 1 g tissue protein that catalyze and decompose 1 μmol NADH in 1 minutes at 25°C is defined as 1 unit.

$$\text{GOGAT activity (U/gprot)}$$

$$= \Delta A_{340} \div (\varepsilon \times d) \times V_{total} \div V_{sample} \div C_{pr} \div T \times f \times 10^6$$

Note:

ΔA_{340}: $A_1 - A_2$.

ε:The molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm

d:Optical path, 0.65 cm

V_{total}:The total volume of the reaction system, 0.22 mL.

V_{sample}:The volume of the sample, 0.02 mL.

C_{pr}: Concentration of protein in sample, gprot/L.

T: The time of reaction, 4 min.

f: Dilution factor of sample before test.

10^6: 1 mol/L=1×10^6 μmol

Appendix I Data

▲Example analysis

For rat liver tissue, dilute the sample of 10% rat liver tissue homogenate for 4 times with reagent 1, take 20 uL of the diluted sample, and carry the assay according to the operation table.

The results are as follows:

the A_1 of the sample is 0.875, after 4 minutes of reaction, the A_2 of the sample is 0.842, the concentration of protein in sample is 12.96 gprot/L, and the calculation result is:

GOGAT activity (U/gprot)

$=(0.875 - 0.842) \div (6220 \times 0.65) \times 0.22 \div 0.02 \div 4 \times 4 \times 10^6 = 6.93$ U/gprot