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

Catalog No：E-BC-K853-M

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

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.036–2.0 mmol/L

Elabscience® Glutamine (Gln) Colorimetric 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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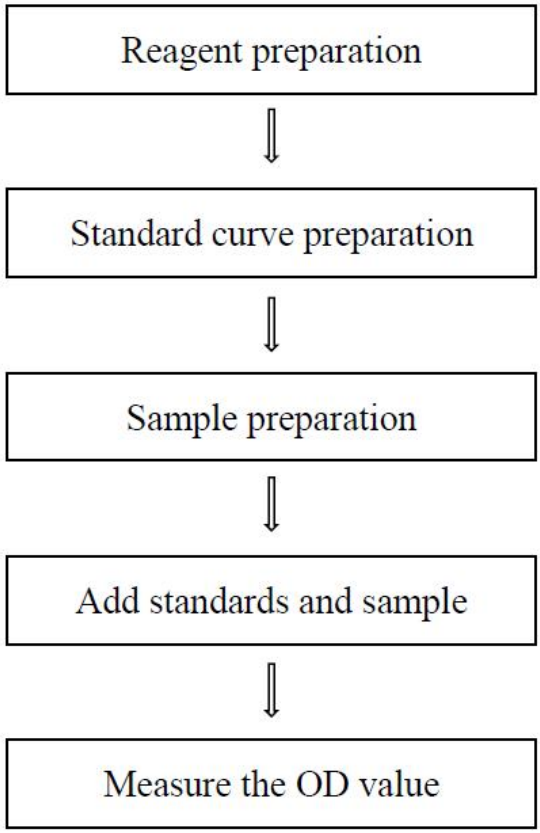
Assay summary



Intended use

This kit can measure glutamine (Gln) content in serum (plasma), tissue, cells and cell culture supernatant samples.

Detection principle

Gln is hydrolyzed to produce glutamic acid under the action of glutaminase. Glutamic acid is further catalyzed by glutamic acid dehydrogenase. Meanwhile, NAD^+ is reduced to NADH, Which under the action of hydrogen transmitter, transfer electrons to WST-8 to produce the yellow product. The content of Gln can be calculated by measuring the change of absorbance value at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Reagent A	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 2	Enzyme Reagent B	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 3	Enzyme Diluent	12 mL×1 vial	24 mL×1 vial	-20℃, 12 months
Reagent 4	Accelerator	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 5	Substrate	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 6	Chromogenic Agent	1.5 mL×1 vial	1.5 mL×2 vials	-20℃, 12 months, shading light
Reagent 7	Standard	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 8	Buffer Solution	3 mL×1 vial	6 mL×1 vial	-20℃, 12 months
	Microplate	48 wells	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:

Incubator, 50 KD Ultrafiltration tube, Microplate reader (440-460 nm, optimum wavelength: 450 nm)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of enzyme reagent A stock solution:
Dissolve one vial of enzyme reagent A with 200 μL of buffer solution, mix well to dissolve. Keep reagent A stock solution on ice during use. Store at -20°C for 3 days protected from light.

③ Preparation of enzyme reagent A working solution:
For each well, prepare 30 μL of enzyme reagent A working solution (mix well 3 μL of enzyme reagent A stock solution and 27 μL of buffer solution). The enzyme reagent A working solution should be prepared on spot and used up within 4 hours.

④ Preparation of enzyme reagent B working solution:
Dissolve one vial of enzyme reagent B with 200 μL of double distilled water, mix well to dissolve. Keep enzyme reagent B working solution on ice during use. Store at -20°C for 3 days protected from light.

⑤ Preparation of accelerator working solution:
Dissolve one vial of accelerator with 1 mL of double distilled water, mix well to dissolve. Store at -20°C for 3 days protected from light.

⑥ Preparation of substrate stock solution：

Dissolve one vial of substrate with 0.5 mL of double distilled water, mix well to dissolve. Store at -20°C for 3 days protected from light.

⑦ Preparation of substrate working solution：

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 1200 μL of substrate working solution (mix well 60 μL of substrate stock solution, 1080 μL of enzyme diluent and 60 μL of accelerator working solution). The substrate working solution should be prepared on spot and used up within 12 hours.

⑧ Preparation of reaction working solution：

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 1400 μL of reaction working solution (mix well 8 μL of enzyme reagent B working solution, 1192 μL of substrate working solution and 200 μL of chromogenic agent). The reaction working solution should be prepared on spot and used up within 1 hour.

⑨ Preparation of 100 mmol/L standard solution：

Dissolve one vial of standard with 1 mL of normal saline (0.9% NaCl), mix well to dissolve. Store at -20°C for 3 days protected from light.

⑩ Preparation of 2 mmol/L standard solution：

Before testing, please prepare sufficient 2 mmol/L standard solution according to the test wells. For example, prepare 1000 μL of 2 mmol/L standard solution (mix well 20 μL of 100 mmol/L standard solution and 980 μL of normal saline (0.9% NaCl)). The 2 mmol/L standard solution should be prepared on spot and used up within the same day.

⑪ The preparation of standard curve：

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

Dilute 2 mmol/L standard solution with normal saline (0.9%NaCl) to a serial

concentration. The recommended dilution gradient is as follows: 0, 0.4, 0.6, 0.8, 1.2, 1.6, 1.8, 2.0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.4**	**0.6**	**0.8**	**1.2**	**1.6**	**1.8**	**2.0**
2 mmol/L standard (μL)	0	40	60	80	120	160	180	200
Normal saline (0.9%NaCl) (μL)	200	160	140	120	80	40	20	0

Sample preparation

① Sample preparation

Serum and plasma: Centrifuge the sample with a 50 KD ultrafiltration tube at 10000×g for 15 min, and collect filtrate on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 0.1 g).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 0.1 g tissue in 900 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 15 minutes to remove insoluble material. Then take the supernatant for centrifugation with a 50 kD ultrafiltration tube at 10000×g for 15 min, and collect filtrate 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 PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 15 minutes to remove insoluble material. Then take the supernatant for centrifugation with a 50 kD ultrafiltration tube at 10000×g

for 15 min, and collect filtrate 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
10% Rat liver tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Rat spleen tissue homogenate	3-5
10% Epipremnum aureum tissue homogenate	3-5
1×10^6 THP-1 cell	1
2×10^6 Molt-4 cell	1
Rat plasma	1
Mouse serum	1
Mouse plasma	1
Human serum	2-3

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Be careful to avoid bubbles when adding reaction working solution.

② The reaction process should be with shading ligh.

③ Add enzyme reagent A working solution at the bottom of microplate reader.

Operating steps

① Standard well: Add 30 μL of enzyme reagent A working solution to the corresponding wells.

Sample well: Add 30 μL of enzyme reagent A working solution to the corresponding wells.

② Add 50 μL of standard solution with different concentrations to standard well. Add 50 μL of sample to sample well.

③ Mix fully with microplate reader for 3 s and incubate at 37℃ for 20 min with shading light.

④ Add 140 μL of reaction working solution to each well.

⑤ Mix fully with microplate reader for 3 s. Measure the OD value of each well at 450 nm with microplate reader, recorded as A_1.

⑥ Incubate at 37℃ for 30 min with shading light.

⑦ Measure the OD value of each well at 450 nm with microplate reader, recorded as A_2, $\triangle A = A_2 - A_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD 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/Plasma and cell culture supernatant:

$$\text{Gln content (mmol/L)} = (\Delta A_{450} - b) \div a \times f$$

2. Tissue sample:

$$\text{Gln content (mmol/kg wet weight)} = (\Delta A_{450} - b) \div a \times f \div (m \div V)$$

3. Cells sample:

$$\text{Gln content (μmol/10^6)} = (\Delta A_{450} - b) \div a \times f \div (n \div V)$$

[Note]

ΔA_{450}: $\Delta A_{Sample} - \Delta A_{Blank}$ (The change of OD value when standard concentration is 0)

m: The weight of wet tissue, g.

n: The number of cells, 10^6 .

V: The volume of homogenate, mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human 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 (mmol/L)	0.08	0.75	1.50
%CV	3.5	3.1	3.0

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.08	0.75	1.50
%CV	4.5	4.9	4.7

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 97%.

	Standard 1	Standard 2	Standard 3
Expected Conc. （mmol/L）	0.5	0.9	1.75
Observed Conc. （mmol/L）	0.5	0.9	1.7
recovery rate(%)	99	95	97

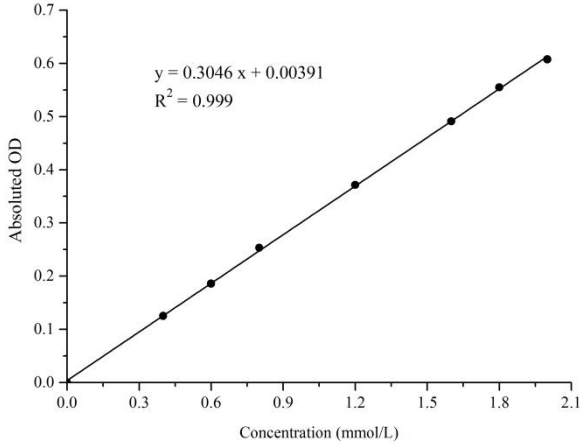
Sensitivity

The analytical sensitivity of the assay is 0.036 mmol/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 OD 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 (mmol/L)	**0**	**0.4**	**0.6**	**0.8**	**1.2**	**1.6**	**1.8**	**2.0**
Average OD	0.017	0.143	0.203	0.271	0.389	0.508	0.572	0.625
Absoluted OD	0	0.126	0.186	0.254	0.371	0.491	0.555	0.608



Appendix II Example Analysis

Example analysis:

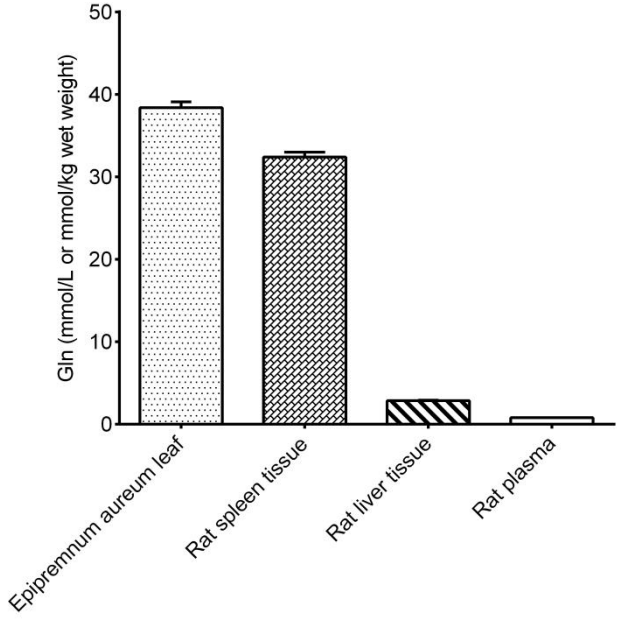
For rat liver tissue, take 50 μL of 10% rat liver tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.2774 x + 0.0046, the average change OD value of the blank (ΔA_{Blank}) is 0.027, the average change OD value of the sample (ΔA_{Sample}) is 0.110, the wet weight of tissue is 0.1g, and the calculation result is:

$$\text{Gln content (mmol/kg wet weight)} = (0.110 - 0.027 - 0.0046) \div 0.2774 \div (0.1 \div 0.9)$$

$$= 2.54 \text{ mmol/kg wet weight}$$

Detect 10% epipremnum aureum tissue homogenate (dilute for 3 times), 10% rat spleen tissue homogenate (dilute for 3 times), 10% rat liver tissue homogenate and rat plasma 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.