PRODUCT INFORMATION & MANUAL

Glutamine Assay Kit (Colorimetric)

NBP3-24497

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

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Glutamine Assay Kit (Colorimetric)

Catalog No: NBP3-24497

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.036 mmol/L

Detection range: 0.036–2.0 mmol/L

Average intra-assay CV (%): 3.2

Average inter-assay CV (%): 4.7

Average recovery rate (%): 97

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

▲Detection principle

Glutamine (Gln) is one of the most abundant amino acids in living organisms. It contains an uncharged amide as a side chain, and is a non-essential amino acid. It is formed by condensation of glutamate and ammonia, but plays an important role in biological processes such as protein synthesis, regulation of acid-balance in mammalian kidneys and cell growth. It is the main source of nitrogen for the synthesis of nucleotides and hexosamine by cells.

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	Specification	Storage
Reagent 1	Enzyme Reagent A	Power × 2 vials	-20℃ , 12 months, shading light
Reagent 2	Enzyme Reagent B	Power × 2 vials	-20℃ , 12 months, shading light
Reagent 3	Enzyme Diluent	24 mL× 1 vial	-20℃ , 12 months
Reagent 4	Accelerator	Power × 1 vial	-20℃ , 12 months, shading light
Reagent 5	Substrate	Power × 2 vials	-20℃ , 12 months, shading light
Reagent 6	Chromogenic Agent	1.5 mL×2 vials	-20℃ , 12 months, shading light
Reagent 7	Standard	Power × 1 vial	-20℃ , 12 months, shading light
Reagent 8	Buffer Solution	6 mL× 1 vial	-20℃ , 12 months
	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

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

Reagents:

Normal saline (0.9% NaCl)

⚠ 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. Be careful to avoid bubbles when adding reaction working solution.
2. The reaction process should be with shading light.
3. Add reagent 1 working solution at the bottom of microplate reader.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 1 stock solution:

   Dissolve a vial of reagent 1 with 200 μL reagent 8 and preserve it on ice for use. The prepared solution can be stored at -20℃ with shading light for 3 days.

   Preparation of reagent 1 working solution:

   Mix the reagent 1 stock solution and reagent 8 at the ratio of 1:9 fully. Prepare the fresh needed amount before use and the prepared solution should be used in 4 h.
3. Preparation of reagent 2 working solution:

   Dissolve a vial of reagent 2 with 200 μL double distilled water and preserve it on ice for use. The prepared solution can be stored at -20℃ with shading light for 3 days.
4. Preparation of reagent 4 working solution:

   Dissolve a vial of reagent 4 with 1 mL double distilled water. The prepared solution can be stored at -20℃ with shading light for 3 days.
5. Preparation of reagent 5 stock solution:

   Dissolve a vial of reagent 5 with 0.5 mL double distilled water. The prepared solution can be stored at -20℃ with shading light for 3 days.

   Preparation of reagent 5 working solution:

   Mix the reagent 5 stock solution, reagent 3 and reagent 4 working solution at the ratio of 1:18:1 fully. Prepare the fresh needed amount before use and the prepared solution should be used in 12 h.

6. Preparation of reaction working solution:

Mix the reagent 2 working solution, reagent 5 working solution and reagent 6 at the ratio of 8:1192:200 fully and preserve it on ice with shading light for detection. Prepare the fresh needed amount before use and the prepared solution should be used in 1 h. (Recommend to prepare during enzymatic reaction).

7. Preparation of 100 mmol/L standard solution:

Dissolve a vial of reagent 7 power with 1 mL normal saline (0.9%NaCl). Prepare fresh needed amount solution before use and the prepared solution can be stored at -20℃ with shading light for 3 days.

Preparation of 2 mmol/L standard solution:

Mix the 100 mmol/L standard solution and normal saline (0.9%NaCl) at the ratio of 1:49 fully. The prepared solution can be used in 1 day.

▲ Sample preparation

1. Serum (plasma) and other liquid sample:

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

2. Tissue sample:

Accurately weigh the tissue, add normal saline (0.9% NaCl) at a ratio of weight (g): volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, 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.

3. Cell sample:

Add homogenization medium at a ratio of cell number (1×10^6): normal saline (0.9% NaCl) (μL) =1: 200, and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, 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.

▲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 (0.036–2.0 mmol/L).

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).

Assay protocol

▲Plate set up

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

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

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:

Number	Standard concentrations (mmol/L)	2 mmol/L standard solution (μL)	Normal saline (μL)
A	0.0	0	200
B	0.4	40	160
C	0.6	60	140
D	0.8	80	120
E	1.2	120	80
F	1.6	160	40
G	1.8	180	20
H	2.0	200	0

2. The measurement of samples

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

Sample well: Add 30 μL of reagent 1 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, $\Delta A = A_2 - A_1$.

▲Summary operation table

	Standard well	Sample well
Reagent 1 working solution (μL)	30	30
Standard solution with different concentrations (μL)	50	
Sample (μL)		50
Mix fully and incubate at 37℃ for 20 min with shading light.		
Reaction working solution (μL)	140	140
Mix fully. Measure the OD value of each well, recorded as A_1.		
Incubate at 37℃ with shading light for 30 min. Measure the OD value of each well, recorded as A_2, $\Delta A = A_2 - A_1$.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum/plasma and cell culture supernatant sample:

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

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:

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

Note:

y: $\Delta A_{Standard} - \Delta A_{Blank}$ (ΔA_{Blank} is the change of OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{450}: $\Delta A_{Sample} - \Delta A_{Blank}$($\Delta A_{Blank}$ is the change of OD value when the 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 tested.

Appendix I Data

▲Example analysis

For rat liver tissue, take 50 μL of 10% rat liver tissue homogenate, and carry the assay according to the operation table.

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:

Gln content (mmol/kg wet weight) = (0.110 - 0.027 - 0.0046) ÷ 0.2774 ÷ (0.1 ÷ 0.9) = 2.54 mmol/kg wet weight