PRODUCT INFORMATION & MANUAL

Glutaminase Activity Assay Kit (Colorimetric)

NBP3-24496

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Glutaminase Activity Assay Kit

Catalog No: NBP3-24496

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.003 U/L

Detection range: 0.003-18.0 U/L

Average intra-assay CV (%): 5.0

Average inter-assay CV (%): 8.3

- ▲ 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 glutaminase activity in serum (plasma), animal and plant tissue samples.

▲Detection principle

Glutaminase (GLS) is an enzyme that catalyzes the hydrolysis of L-β -glutamine to L- glutamate and ammonia. In human body, glutaminase is mainly related to cell growth and metabolism, which can regulate acid-base balance and maintain the stability of internal environment. Glutaminase is located at the base of mitochondrial intima, and can inhibit the metabolism of glutamine in vivo by decreasing the activity of glutaminase in tumor cells clinically, so as to inhibit the growth of tumor cells.

Glutamine is decomposed to produce glutamic acid under the action of glutaminase. Glutamic acid is further transformed 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 activity of glutaminase can be calculated by measuring the change of absorbance value at 450 nm.

▲Kit components & storage

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

Test tube, Incubator, Centrifuge, Microplate reader (440-460 nm, optimum wavelength: 450 nm)

Reagents:

PBS (0.01 mol/L, pH 7.4)

⚠ 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. To avoid contamination, it is recommended to aliquot the reagent 7 working solution into smaller quantities.
2. The incubation process of chromogenic reaction should be with shading light.

Pre-assay preparation

▲ Reagent preparation

1. Preserve reagent 4 on ice for detection, and bring other reagents to room temperature before use.
2. Preparation of reagent 1 working solution:
   Dissolve a vial of reagent 1 powder with 5 mL double distilled water and mix fully. The prepared solution can be stored at -20℃ for 3 days.
3. Preparation of 50 mmol/L standard stock solution:
   Dissolve a vial of reagent 2 with 1 mL reagent 3 and mix fully. The prepared solution can be stored at 2-8℃ for 3 days.
   Preparation of 0.5 mmol/L standard solution:
   Dilute 50 mmol/L standard stock solution with double distilled water at a ratio of 1:99 and mix fully. Prepare the fresh needed amount before use.
4. Preparation of reagent 4 working solution:
   Dissolve a vial of reagent 4 with 200 uL double distilled water and preserve it on ice for detection. The prepared solution can be stored at 2-8℃ for 6 h with shading light.
5. Preparation of reagent 6 working solution:
   Dissolve a vial of reagent 6 with 0.4 mL reagent 3 and mix fully. The prepared solution can be stored at -20℃ for 3 days with shading light, avoid repeated freezing and thawing.
6. Preparation of reagent 7 working solution:
   Dissolve a vial of reagent 7 with 1 mL double distilled water and mix fully. The prepared solution can be stored at -20℃ for 3 days with shading light.
7. Preparation of reaction working solution:
   Mix the reagent 4 working solution, reagent 5, reagent 6 working solution and reagent 7 working solution at the ratio of 10:690:37:10 fully and preserve it on ice for detection. Prepare the fresh needed amount before use and the prepared solution should be used in 1 h.

▲ Sample preparation

1. Serum and plasma samples:

   Detect the sample directly. If the sample is turbidity, centrifuge at 8000 g for 10 min, then take the supernatant for detection.

2. Tissue sample:

   Accurately weigh the tissue, add PBS (0.01 mol/L, pH 7.4) 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 detection. Meanwhile, determine the protein concentration of supernatant. If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use.

▲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 (0.003-18.0 U/L).

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat kidney tissue homogenate	1
Human serum	1
Human plasma	1-3

Note: The diluent is PBS (0.01 mol/L, pH 7.4).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note:A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲Detailed operation steps

1. The preparation of standard

Dilute 0.5 mmol/L standard solution with PBS to a serial concentration. The recommended dilution gradient is as follows: 0.0, 0.1, 0.15, 0.2, 0.3, 0.4, 0.45, 0.5 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	0.5 mmol/L standard solution (μL)	PBS (μL)
A	0	0	200
B	0.1	40	160
C	0.15	60	140
D	0.2	80	120
E	0.3	120	80
F	0.4	160	40
G	0.45	180	20
H	0.5	200	0

2. The measurement of samples

2.1 Enzymatic reaction

(1) Sample tube: Take 20 μL of sample to the 1.5 mL EP tube.

Control tube: Take 20 μL of sample to the 1.5 mL EP tube.

(2) Add 80 μL of regent 1 working solution to the sample tube, and add 80 μL of double distilled water to the control tube.

(3) Mix fully and incubate at 37℃ for 30 min.

(4) Centrifuge at 8000 g for 5 min at room temperature. The supernatant is used for chromogenic reaction.

2.2. Chromogenic reaction

(1) Standard well: Take 50 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Take 50 μL supernatant of sample tube to the corresponding wells.

Control well: Take 50 μL supernatant of control tube to the corresponding wells.

(2) Add 140 μL of reaction working solution to each well.

(3) Add 20 μL of regent 8 to each well.

(4) Mix fully with microplate reader and incubate at 37℃ for 20 min with shading light. Measure the OD value of each well at 450 nm with microplate reader.

▲Summary operation table

1. Enzymatic reaction

	Sample tube	Control tube
Sample (μL)	20	20
Double distilled water (μL)		80
Regent 1 working solution (μL)	80	
Mix fully and incubate at 37℃ for 30 min.		
Regent 2 (μL)	20	20
Centrifuge at 8000 g for 5 min at room temperature. The supernatant is used for chromogenic reaction.		

2. Chromogenic reaction

	Standard well	Sample well	Control well
Standard solution with different concentrations (μL)	50		
Supernatant of sample tube (μL)		50	
Supernatant of control tube (μL)			50
Reaction working solution (μL)	140	140	140
Regent 8 (μL)	20	20	20
Mix fully and incubate at 37℃ for 20 min with shading light. Measure the OD value of each well.			

▲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. Tissue sample:

Definition: The amount of GLS in 1 g tissue protein per 1 minute that hydrolyze the glutamine to produce 1 μmol glutamic acid at 37°C is defined as 1 unit.

$$\text{GLS activity (U/gprot)} = (\Delta A_{450} - b) \div a \div T \div C_{pr} \div (V_1 \div V_2) \times f \times 1000^*$$

2. Serum/plasma sample:

Definition: The amount of GLS in 1 L liquid sample per 1 minute that hydrolyze the glutamine to produce 1 μmol glutamic acid at 37°C is defined as 1 unit.

$$\text{GLS activity (U/L)} = (\Delta A_{450} - b) \div a \div T \div (V_1 \div V_2) \times f \times 1000^*$$

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the 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}: OD_{Sample} – $OD_{Control.}$

T: The time of enzymatic reaction, 30 min.

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

V_1: The volume of enzymatic reaction solution added to the chromogenic reaction system, 50 μL.

V_2: Total volume of enzymatic reaction, 100 μL.

f: Dilution factor of sample before tested.

1000*: 1 mmol/L =1000 μmol/L.

Appendix I Data

▲Example analysis

For rat liver tissue, take 20 μL of 10% rat liver tissue homogenate, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 1.1458 x - 0.002, the average OD value of the control is 0.103, the average OD value of the sample is 0.136, the concentration of protein in sample is 6.58 gprot/L, and the calculation result is:

GLS activity (U/gprot) = (0.136 – 0.103 + 0.002) ÷ 1.1458 ÷ 30 ÷ 6.58 ÷ (50 ÷ 100) × 1000 = 0.309 U/gprot

Appendix II References

1. Szeliga M, Matyja E, Obara M, et al. Relative Expression of mRNAS Coding for Glutaminase Isoforms in CNS Tissues and CNS Tumors [J]. Neurochemical Research, 2008, 33(5):808-813.
2. Wise D R, Deberardinis R J, Mancuso A, et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction [J]. Proceedings of the National Academy of Sciences of the United States of America, 2008, 105(48):18782-18787.