TECHNICAL MANUAL

Glutaminase (GLS) Activity Assay Kit

- **SKU CODE:** MAES0230
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Enzymatic reaction
- Chromogenic reaction
- Measure absorbance at 450 nm

2. Intended use

This kit can measure glutaminase activity in serum (plasma), animal and plant tissue samples.

3. Detection principle

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, transfers electrons to WST-8 to produce a yellow product. The activity of glutaminase can be calculated by measuring the change of absorbance value at 450 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Substrate A	Powder × 2 vials	-20°C, 12 months
Reagent 2	Standard	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 3	Diluent	4 mL×1 vial	-20°C, 12 months
Reagent 4	Enzyme Reagent	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 5	Buffer Solution	20 mL×1 vial	-20°C, 12 months
Reagent 6	Substrate B	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 7	Accelerator	Powder × 2 vials	-20°C, 12 months, shading light

Item	Component	Size (96 T)	Storage
Reagent 8	Chromogenic Agent	1.5 mL × 2 vials	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Keep enzyme reagent on ice during use. Equilibrate other reagents to room temperature before use.
2. **Preparation of substrate A working solution:** Dissolve one vial of substrate A with 5 mL of double distilled water, mix well to dissolve. Store at -20°C for 3 days.
3. **Preparation of 50 mmol/L standard stock solution:** Dissolve one vial of standard with 1 mL of diluent, mix well to dissolve. Store at 2-8°C for 3 days.
4. **Preparation of 0.5 mmol/L standard solution:** Before testing, please prepare sufficient 0.5 mmol/L standard solution according to the test wells. For example, prepare 1000 µL of 0.5 mmol/L standard solution (mix well 10 µL of 50 mmol/L standard stock solution and 990 µL of double distilled water). The 0.5 mmol/L standard solution should be prepared on spot.
5. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 200 µL of double distilled water, mix well to dissolve. The enzyme working solution should be stored at 2-8°C and used up within 6 hours.
6. **Preparation of substrate B working solution:** Dissolve one vial of substrate B with 400 µL of diluent, mix well to dissolve. Aliquot and store at -20°C for 3 days protected from light, and avoid repeated freeze/thaw cycles.

7. **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.
8. **Preparation of reaction working solution:** Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 747 µL of reaction working solution (mix well 10 µL of enzyme working solution, 690 µL of buffer solution, 37 µL of substrate B working solution and 10 µL of accelerator working solution). Keep reaction working solution on ice during use. The reaction working solution should be prepared on spot and used up within 1 hour.
9. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 0.5 mmol/L standard solution with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 0.1, 0.15, 0.2, 0.3, 0.4, 0.45, 0.5 mmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

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 PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

4. Centrifuge at 10000×g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

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 M, pH 7.4). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. To avoid contamination, it is recommended to aliquot the accelerator working solution into smaller quantities.
2. The incubation process of chromogenic reaction should be protected from light.

9. Operating steps

Enzymatic reaction:

1. Sample well: 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 substrate A 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°C for 30 min.

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

Chromogenic reaction:

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

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

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

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

3. Add 20 μL of chromogenic agent to each well.

4. Mix fully with microplate reader for 3 s and incubate at 37°C for 20 min with shading light. Measure the OD value of each well at 450 nm with microplate reader.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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) sample:

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

GLS activity (U/L) = (ΔA_450 - b) ÷ a ÷ T ÷ (V_1 ÷ V_2) × f × 1000*

2. Tissue sample:

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

GLS activity (U/gprot) = (ΔA_450 - b) ÷ a ÷ T ÷ C_{pr} ÷ (V_1 ÷ V_2) × f × 1000*

[Note]

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 test.

1000*: 1 mmol/L = 1000 μmol/L

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.26	2.80	13.50
%CV	5.4	5.2	4.7

Inter-assay Precision

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

Inter-assay Precision

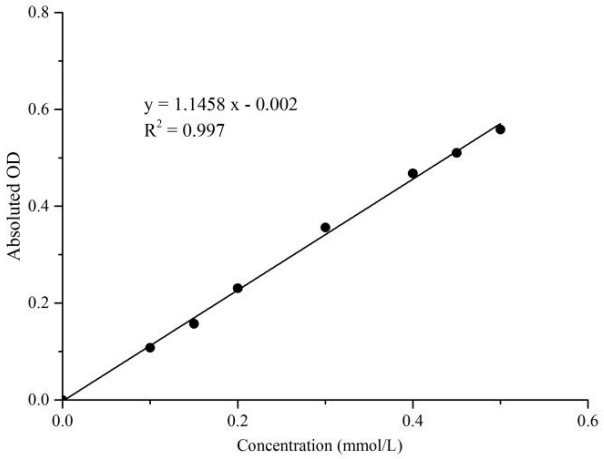
Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.26	2.80	13.50
%CV	8.2	7.9	8.8

Sensitivity

The analytical sensitivity of the assay is 0.003 U/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.

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), the standard curve and data are provided below for reference only:



Standard curve

Concentration (mmol/L)	Average OD	Absolute OD
0	0.067	0.000
0.1	0.175	0.108
0.15	0.224	0.158
0.2	0.298	0.231
0.3	0.423	0.357
0.4	0.535	0.468
0.45	0.577	0.511
0.5	0.625	0.559

12. Appendix II Example Analysis

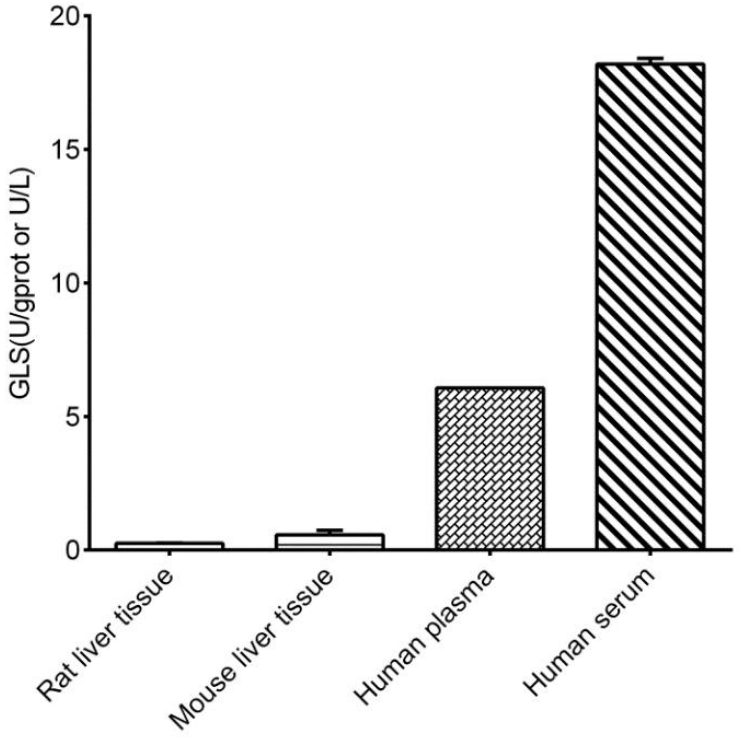
Example analysis:

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

Detect 10% rat liver tissue homogenate (the concentration of protein is 6.58 gprot/L), 10% mouse liver tissue homogenate (the concentration of protein is 6.68 gprot/L), human plasma and human serum according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.