Product Manual

Glutamine Assay Kit (Colorimetric)

Catalog Number

MET-5166 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Glutamine is a conditionally essential amino acid (needed from the diet under stress conditions) that is used in protein biosynthesis. Its side chain is almost identical to glutamic acid, except the carboxylic acid group on the side chain is replaced by an amide group. Glutamine is the most abundant free amino acid in human blood. Many food sources are rich in glutamine including protein-rich foods such as dairy products, eggs, fish, chicken, beef, as well as vegetables like beans, beets, cabbage, spinach, and carrots. In addition to protein synthesis/function, glutamine plays a role in acid-base balance in the kidney by producing ammonium, as a source of cellular energy next to glucose, and as a nitrogen donator for many anabolic processes such as the synthesis of purine. Cancer cells rely on glutamine as a nitrogen and carbon source. The importance of glutamine in certain tumors has been demonstrated. For example, blocking glutamine metabolism was shown to inhibit growth of several tumors such as breast, liver, kidney and T-cell lymphoblastic leukemia.

Cell Biolabs' Glutamine Assay Kit is a simple assay for measuring glutamine levels in biological samples without any need for pretreatment. The kit has a detection sensitivity limit of 3.13 µM glutamine. Each kit provides sufficient reagents to perform up to 200 assays*, including standard curve and unknown samples.

Note: Each sample replicate requires 2 assays, one treated with Glutaminase (+G) and one without (-G). Glutamine is calculated from the difference in OD readings from the 2 wells.

Assay Principle

The Glutamine Assay Kit is a sensitive quantitative colorimetric assay for glutamate. Glutaminase converts glutamine and H_2O to glutamate and NH_3. Glutamate oxidase converts glutamate to α-ketoglutarate and NH_3 as well as H_2O_2. L-alanine and glutamate-pyruvate transaminase are also added to the reaction in order to regenerate glutamate and H_2O_2. As a result, multiple rounds of the reaction occur which results in significant amplification of H_2O_2 production. The H_2O_2 is then detected with a highly specific colorimetric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples and standards are incubated for 30 minutes and then read with a standard 96-well colorimetric plate reader (Figure 1). Samples are compared to a known concentration of glutamine standard within the 96-well microtiter plate format.

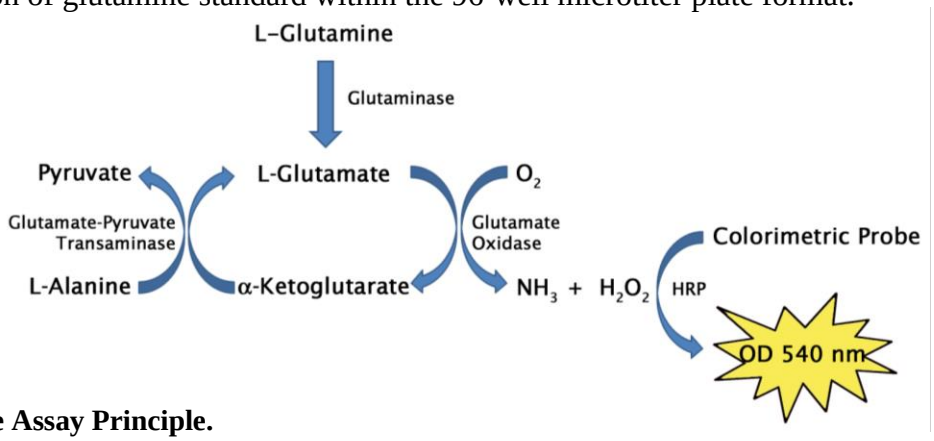

Figure 1. Glutamine Assay Principle.

Related Products

1. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
2. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit

3. STA-341: OxiSelect™ Catalase Activity Assay Kit
4. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit

Kit Components (shipped on blue ice)

1. Glutaminase (Part No. 51651D): One 1 mL vial at 2.5 U/mL.
2. Negative Control Buffer (Part No. 51652B): One 1 mL vial.
3. Glutamate Oxidase (Part No. 267402): One 160 µL vial at 5 U/mL.

Note: One unit is defined as the amount of enzyme that will form 1.0 micromole of alpha-ketoglutaric acid from L-glutamic acid per minute at pH 7.4 at 30°C.

4. Glutamate-Pyruvate Transaminase (Part No. 267403): One 50 µL vial at 100 U/mL.

Note: One unit is defined as the amount of enzyme that will cause the transamination of 1.0 µmole of L-alanine per minute at pH 7.5 and 25°C.

5. L-Alanine (Part No. 267404): One 10 µL vial at 200 mM.
6. L-Glutamine Standard (Part No. 51653B): One 100 µL vial at 10 mM.
7. Colorimetric Probe (Part No. 268003): One 250 µL amber tube of a 10 mM solution in DMSO.
8. HRP (Part No. 234402): One 100 µL tube.
9. 5X Assay Buffer (Part No. 51654A): One 50 mL bottle of 1 M sodium acetate pH 4.9.
10. 5X Reaction Buffer (Part No. 51655A): One 50 mL bottle of 1 M Tris pH 8.0.

Materials Not Supplied

1. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate

Storage

Upon receipt, store the 5X Assay Buffer and 5X Reaction Buffer at room temperature. Aliquot and store Glutaminase, Glutamate Oxidase, and Glutamate-Pyruvate Transaminase at -80°C. Aliquot and store all other components at -20ºC. Avoid multiple freeze/thaw cycles. The Colorimetric Probe is light sensitive and must be stored accordingly.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer and 1X Reaction Buffer: Dilute the stock 5X Assay Buffer and 5X Reaction Buffer 1:5 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Reaction Mix: Prepare mixture according to the table below. The Reaction Mix is stable for 1 day at 4ºC. *Note: Scale down the described example appropriately and prepare only enough for immediate use*.

Component	Reaction Mix (20 assays)
Colorimetric Probe	10 µL
HRP	2 µL
Glutamate Oxidase	16 µL
Glutamate-Pyruvate Transaminase	5 µL
L-Alanine	1 µL
1X Reaction Buffer	966 µL
Total	**1000 µL**

Preparation of Samples

- Cell culture supernatants: To remove insoluble particles, centrifuge at 10,000 x g for 10 min at 4°C. Collect the supernatant and filter the solution with a 10 kDa spin filter to deproteinate the sample. Collect flow through. The flow through can be assayed directly or diluted as necessary into distilled water. Prepare the Glutamine standard curve in the same non-conditioned deproteinated media.

Note: Maintain pH between 7 and 8 for optimal working conditions as the Colorimetric Probe is unstable at high pH (>8.5).

- Cell lysates: Resuspend cells in distilled water. Homogenize or sonicate the cells on ice. Centrifuge 10,000 x g for 10 minutes at 4°C to remove debris. Collect the supernatant and filter the solution with a 10 kDa spin filter to deproteinate the sample. Collect flow through. The flow through may be assayed undiluted or diluted as necessary into distilled water.
- Tissue lysates: Sonicate or homogenize tissue sample in distilled water and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect the supernatant and filter the solution with a 10 kDa spin filter to deproteinate the sample. Collect flow through. The flow through may be assayed directly or diluted as necessary into distilled water.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 x g for 10 min at 4°C. Collect the supernatant and filter the solution with a 10 kDa spin filter to deproteinate the sample. Collect flow through. The flow through can be assayed directly or diluted as necessary into distilled water.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Colorimetric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since Resorufin is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Glutamine standards before use by diluting in distilled water according to Table 1 below.

Standard Tubes	10 mM Glutamine Solution (µL)	Distilled Water (µL)	Glutamine (µM)
1	10	490	200
2	250 of Tube #1	250	100
3	250 of Tube #2	250	50
4	250 of Tube #3	250	25
5	250 of Tube #4	250	12.5
6	250 of Tube #5	250	6.25
7	250 of Tube #6	250	3.13
8	0	250	0

Table 1. Preparation of Glutamine Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

Note: Each unknown sample replicate requires two paired wells, one to be treated with Glutaminase (+G) and one without the enzyme (-G) to measure endogenous background.

2. Add 25 µL of each Glutamine Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 25 µL of 1X Assay Buffer to all wells and mix the well contents thoroughly.
4. Add 5 µL of Glutaminase *(+G)* to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
5. Add 5 µL of Negative Control Buffer *(-G)* to the other half of the paired sample wells.
6. Mix the well contents thoroughly and incubate for 30 minutes at 37ºC protected from light.
7. Add 50 µL of Reaction Mix to each well.
8. Mix the well contents thoroughly and incubate for 30 minutes at 37ºC protected from light.

Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.

9. Read the plate with a spectrophotometric microplate reader in the 540-570 nm range.

Example of Results

The following figures demonstrate typical Glutamine Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

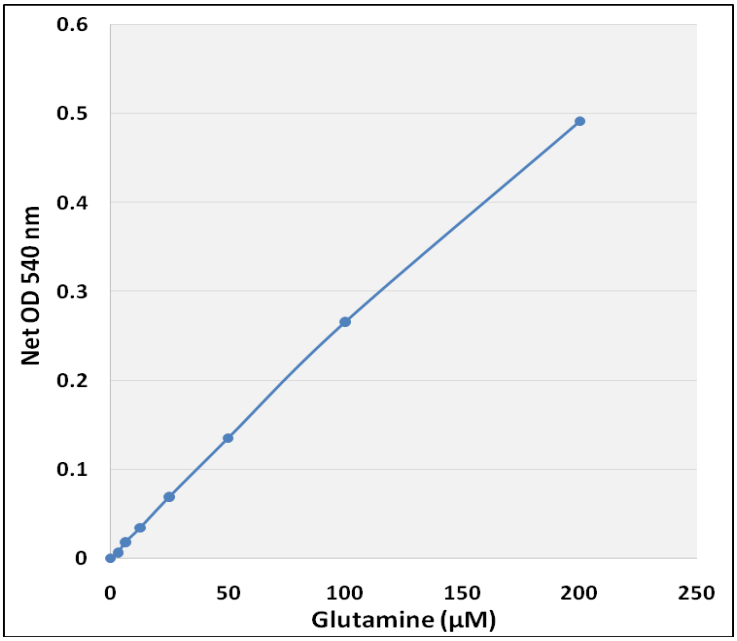

Figure 2. Glutamine Standard Curve.

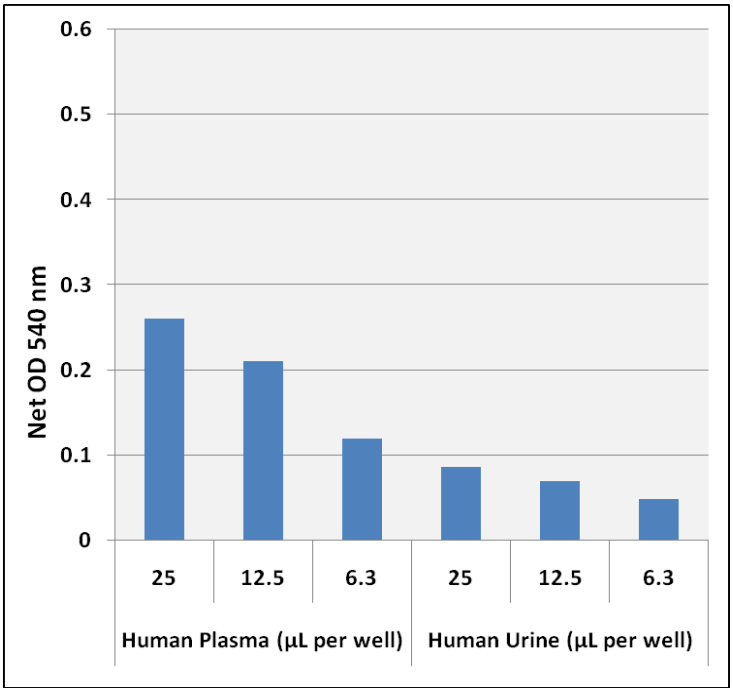

Figure 3. Detection of Glutamine in Human Plasma or Urine. Human plasma or urine was deproteinated according to the preparation of samples section above.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
3. Subtract the average zero standard value from itself and all standard values.
4. Graph the standard curve (see Figure 1).
5. Subtract the sample well values without Glutaminase *(-G)* from the sample well values containing Glutaminase (*+G*) to obtain the difference. The absorbance difference is due to the glutaminase activity:

$$\Delta A = A(+G) - A(-G)$$

6. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of Glutamine present in the sample. Only use values within the range of the standard curve.

References

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Warranty

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