Glutamine Assay Kit

Glutamine Assay Kit is a detection kit for the quantification of Glutamine in Urine, Serum, Plasma, Tissue extracts, Cell lysate, and Cell culture media.

Catalog number: ARG83048

Package: 96 wells

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INTRODUCTION

Glutamine (symbol Gln or Q)is an α-amino acid that is used in the biosynthesis of proteins. Its side chain is similar to that of glutamic acid, except the carboxylic acid group is replaced by an amide. It is classified as a charge-neutral, polar amino acid. It is non-essential and conditionally essential in humans, meaning the body can usually synthesize sufficient amounts of it, but in some instances of stress, the body's demand for glutamine increases, and glutamine must be obtained from the diet.It is encoded by the codons CAA and CAG.

In human blood, glutamine is the most abundant free amino acid.

The dietary sources of glutamine include especially the protein-rich foods like beef, chicken, fish, dairy products, eggs, vegetables like beans, beets, cabbage, spinach, carrots, parsley, vegetable juices and also in wheat, papaya, Brussels sprouts, celery, kale and fermented foods like miso.

PRINCIPLE OF THE ASSAY

This Glutamine Assay Kit is a simple colorimetric assay that measures the amount of Glutamine in Urine, Serum, Plasma, Tissue extracts, Cell lysate, and Cell culture media. The assay is based on the enzyme driven reaction. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate. The pNA light emission can be quantified using a microtiter plate reader at 420nm. The concentration of Caspase 1 in the samples is then determined by comparing the O.D. 420 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Microplate	1 X 96-well plate	RT
Standard (100 µmol/L)	1 vial	4°C
Assay Buffer	4 x 30 mL (ready to use)	4°C
Reaction Buffer A	10 mL	4°C
Reaction Buffer B	10 mL	4°C
Enzyme	20 µl	4°C
Reaction Dye	2 mL	4°C
Stop Solution	10 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading in the 420 nm range
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

If the enzyme activity is higher, please dilute the sample with PBS, or decrease the reaction time. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time to 2 hours, even overnight.

- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months.

Cell Culture media- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Tissue samples- Weigh out 0.05 g tissue, homogenize with 0.5 mL Assay Buffer-A, 5 µL of Assay Buffer-B and 5 µL of Reducing Agent on ice for 10 minutes. Centrifuged at 1,0000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection. Assay samples immediately or aliquot and store samples at ≤ -20°C (store at -80°C is recommended) for up to one month. Avoid repeated freeze-thaw cycles.

Note: BCA method is not suitable for the determination of protein concentration. It is better to use Bradford method.

REAGENT PREPARATION

- Enzyme: Add 1 mL of Reaction Buffer A to dissolve before use. Mix thoroughly to make sure the reagent is dissolved completely before use, and store the reconstituted Enzyme at 4°C.
- Standard: add 1 ml Distilled Water to dissolve, then add 10 µL of standard into 990 µL of Distilled Water, the concentration will be 1 mmol/L.
  Use the 1 mmol/L Standardsto prepare a series of standards.

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate.

1. Add **20 µL** of **Samples** into Sample tube.
2. Add **20 µL** of **Distilled Water** into Control tube.
3. Add **50 µL** of **Reaction Buffer A** into Sample tube and Control tube.
4. Add **10 µL** of **Enzyme** into Sample tube and Control tube.
5. Mix well and incubate plate for **1 hour** at **37°C**.
6. Add **100 µL** of **Stop Solution** into Sample tube and Control tube.
7. Mix well, centrifuged at 8,000g for 5 minutes, add the supernatant into the microplate.
8. Add **90 µL** of **Samples, Control, Standard and Blank** into microplate.
9. Add **90 µL** of **Reaction Buffer B** into All wells.
10. Add **20 µL** of **Reaction Dye** into All wells.
11. Read the plate with a microplate reader at **420 nm**.

Summary of Glutamine Assay Procedure

Reagent	Sample	Control	Standard	Blank
Sample	20 µl	-	-	-
Distilled Water	-	20 µl	-	-
Reaction Buffer A	50 µl	50 µl	-	-
Enzyme	10 µl	10 µl	-	-
Mix well and incubate plate for **1 hour** at **37°C**				
Stop Solution	100 µl	100 µl	-	-
Mix well, centrifuged at 8,000g for 5 minutes, add the supernatant into the microplate.				
Supernatant	90 µl	90 µl	-	-
Standard	-	-	90 µl	-
Distilled water	-	-	-	90 µl
Reaction Buffer B	90 µl	90 µl	90 µl	90 µl
Reaction Dye	20 µl	20 µl	20 µl	20 µl
Read the OD with a microplate reader at **420 nm** immediately.				

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Blank, Control and samples.
2. Calculation:

A. Definition:

$C_{Protein}$: the protein concentration of sample, mg/mL;

W: the weight of sample, g;

N: the quantity of cell, N x 10^4;

$C_{Standard}$: the concentration of standard, 1 mmol/L = 1 μmol/mL;

$V_{Standard}$: the volume of standard, 0.09 mL;

V_{Sample}: the volume of sample, 0.02 mL;

V_{Assay}: the volume of Assay buffer, 1 mL;

B. Formula:

a). According to the protein concentration of sample

Glutamine (μmol/ml)

$= [(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times 2)]$

$= \mathbf{9 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})}$

b). According to the weight of sample

Glutamine (μmol /g)

$= [(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times W / V_{Assay}) \times 2]$

$\mathbf{= 9 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times W]}$

c). According to the quantity of cells

Glutamine ($U/10^4$) =

= $[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times N / V_{Assay}) \times 2]$

= $9 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times N]$

3. Detection range:

   The detection range is from 0.01mmol/L – 1 mmol/L.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and this kit does not need serial diluted standards.