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 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 (50 µmol)	1 vial	4°C
Assay Buffer	4 x 30 mL (ready to use)	4°C
Reaction Buffer	10 mL	4°C
Enzyme	1 vial	4°C
Reaction Dye A	1 vial	4°C
Reaction Dye B	1 vial	4°C
Reaction Dye B Diluent	4 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading in the 620 nm range
- Centrifuge
- Mortar
- Deionized or Distilled water
- 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 sample, it is recommended to perform a pre-experiment first in order to determine the optimal assay conditions before proceeding with full testing.
- 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.

Cell Culture Supernatant- Collect cell / bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue samples- Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Note: Liquid sample can detect directly.

REAGENT PREPARATION

- **Enzyme:** Add **1 mL** of Reaction Buffer to dissolve before use. Mix thoroughly to make sure the reagent is dissolved completely before use, and store the reconstituted Enzyme at 4°C.
- **Reagent Dye A:** Add **1 mL** of distilled water to dissolve before use. Mix thoroughly to make sure the reagent is dissolved completely before use, and store the reconstituted Enzyme at 4°C.
- **Reagent Dye B:** Add **1 mL** of **Reaction Dye B Diluent** 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** of Distilled Water to dissolve, then add **100 µL** of standard into **400 µL** of Distilled Water, the concentration will be **10 µmol/mL**. Use the **10 µmol/mL** Standards to perform 2-fold serial dilutions to make the standard curve.

ASSAY PROCEDURE

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

1. Add **60 µL** of **Reaction Buffer** into each well.
2. Add **20 µL** of **Samples** into Sample well.
3. Add **20 µL** of **Standard** into Standard well.
4. Add **30 µL** of **Distilled Water** into blank well.
5. Add **10 µL** of **Enzyme** into Sample and Standard well.
6. Add **70 µL** of **Reaction Dye A** into each well.
7. Add **40 µL** of **Reaction Dye B** into each well.
8. Mix well, Incubate the plate at **37°C** for **5 minutes**.
9. Read the plate with a microplate reader at **620 nm**.

Summary of Glutamine Assay Procedure

Reagent	Sample	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl
Sample	20 µl	-	-
Standard	-	20 µl	-
Distilled Water	-	-	30 µl
Enzyme	10 µl	10 µl	-
Reaction Dye A	70 µl	70 µl	70 µl
Reaction Dye B	40 µl	40 µl	40 µl
Mix well, incubate at 37°C for 5 minutes. Read the plate with a microplate reader at 620 nm.			

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, 10 μmol/mL;

   $V_{Standard}$: the volume of standard, 0.02 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 volumeof sample:

   Glutamine **(μmol/ml)**

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

   = **$10 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$**

   b). According to the weight of sample:

   Glutamine **(μmol/g)**

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

   = $10 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times W]$

c). According to the quantity of cell / bacteria:

Glutamine **($\mu mol/10^4$)** =

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

= **$10 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times N]$**

3. Detection range:

   The detection range is from 0.1 µmol/mL – 10 µmol/mL.

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.