



# **Glutamine Microplate Assay Kit**

## **User Manual**

**Catalog # CAK1206**

(Version 2.2C)

Detection and Quantification of Glutamine (Gln) content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture and Other biological fluids Samples.

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

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## I. INTRODUCTION

Glutamine is an amino acid synthesized in the muscle that plays major roles in protein synthesis, acid-base balance, anabolic processes and is utilized for cellular energy and as a carbon source. It is used in treatment of injury, trauma, burns, and also as a supplement for muscle growth and post-surgery healing. Simple, direct and automation-ready procedures for measuring glutamine concentration are very desirable.

Glutamine Microplate Assay Kit is based on hydrolysis of glutamine to glutamate and colorimetric determination of the product. The intensity of the product color, measured at 620 nm, is proportional to the glutamine concentration in the sample.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Enzyme	Powder x 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent II Diluent	4 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

### Note:

**Enzyme:** add 1 ml Reaction Buffer to dissolve before use, store at 4 °C.

**Dye Reagent I:** add 7 ml distilled water to dissolve before use, store at 4 °C.

**Dye Reagent II:** add 4 ml Dye Reagent II Diluent to dissolve before use, store at 4 °C.

**Standard:** add 1 ml distilled water to dissolve before use, then add 100 µl into 400 µl distilled water, mix; the concentration is 10 mmol/L, store at 4 °C.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

#### **IV. SAMPLE PREPARATION**

##### **1. For cell and bacteria samples**

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for  $5 \times 10^6$  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.

##### **2. For 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.

##### **3. For liquid sample.**

Detect directly.

## V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

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	--
Dye Reagent I	70 µl	70 µl	70 µl
Dye Reagent II	40 µl	40 µl	40 µl
Mix, incubate at 37 °C for 5 min, record absorbance measured at 620 nm.			

### Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) The concentrations can vary over a wide range depending on the different samples.  
For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- 3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

1. According to the volume of sample

$$\begin{aligned} \text{Gln } (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} \\ &= 10 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{Gln } (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times W / V_{\text{Assay}}) \\ &= 10 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{Gln } (\mu\text{mol}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times N / V_{\text{Assay}}) \\ &= 10 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / N \end{aligned}$$

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

$C_{\text{Standard}}$ : the concentration of Standard, 10 mmol/L = 10  $\mu\text{mol/ml}$ ;

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

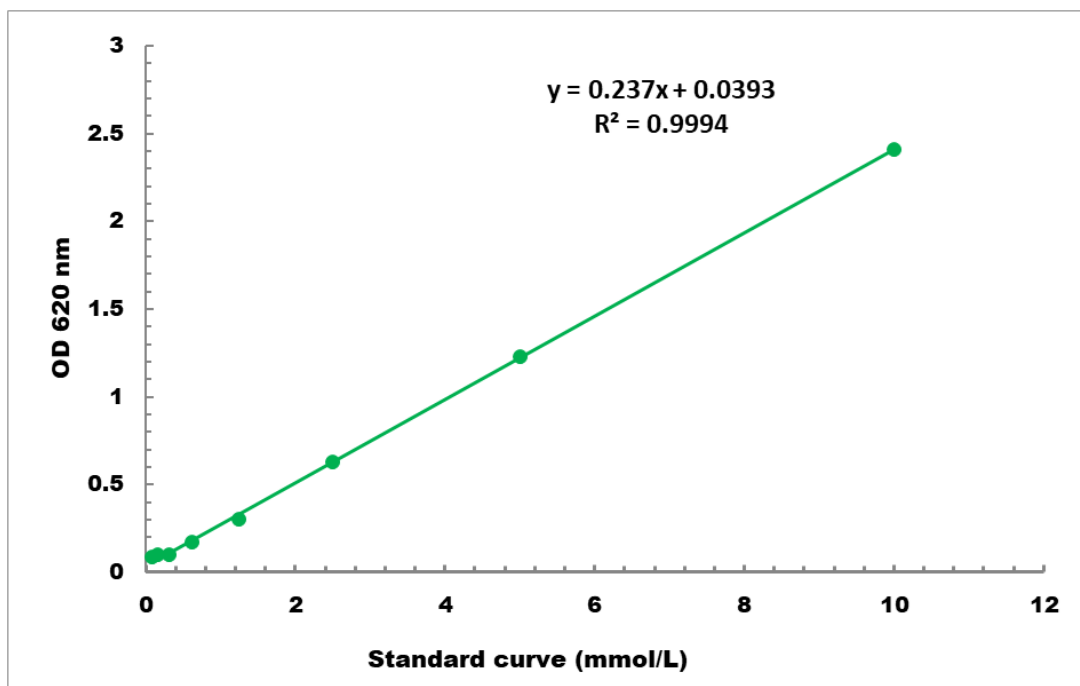
$V_{\text{Sample}}$ : the volume of sample, 0.02 ml;

$V_{\text{Standard}}$ : the volume of sample, 0.02 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer in sample preparation, 1 ml.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 10 mmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES