GlutamineMicroplate Assay Kit

User Manual

Catalog # CAK1206

(Version 1.3A)

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

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

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 420 nm, is proportional to the glutamine concentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Enzyme	20 µlx 1	4 °C
Reaction Buffer I	10 mlx 1	4 °C
Reaction Buffer II	10 mlx 1	4 °C
Stop Solution	10 mlx 1	4 °C
Dye Reagent	2 mlx 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Enzyme: add 1 mlReaction Buffer I to dissolve before use, store at 4 °C.

Standard: add 1 mldistilled water to dissolve before use, then add 10 µl into 990µl distilled water, mix; the concentration is 1 mmol/L, store at 4 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 420 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, 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	Control	Standard	Blank
Sample	20 µl	--	--	--
Distilled water	--	20 µl	--	--
Reaction Buffer I	50 µl	50 µl	--	--
Enzyme	10 µl	10 µl	--	--
Mix, put it in the oven,37°Cfor 1 hour.				
Stop Solution	100 µl	100 µl	--	--
Mix, centrifuged at 8,000g for 5minutes, add the supernatant intothe microplate.				
Supernatant	90 µl	90 µl	--	--
Standard	--	--	90 µl	--
Distilled water	--	--	--	90 µl
Reaction Buffer II	90 µl	90 µl	90 µl	90 µl
Dye Reagent	20 µl	20 µl	20 µl	20 µl
Mix,record absorbance measured at 420 nm immediately.				

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

$$Gln\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times 2$$

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

2. According to the weight of sample

$$Gln\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) \times 2$$

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

3.According to the quantity of cells or bacteria

$$Gln\ (\mu mol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) \times 2$$

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

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

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

W: the weight of sample, g;

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

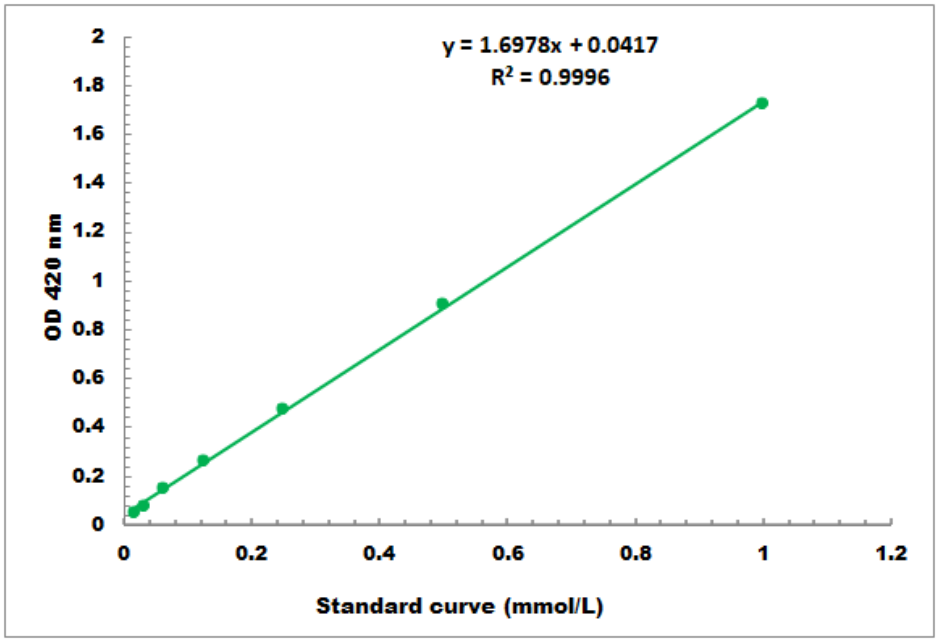
V_{Sample}: the volume of sample, 0.02 ml;

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

V_{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: 0.01 mmol/L -1 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES