



Glutamine Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1206

(Version 2.3D)

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.

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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 Colorimetric 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	30mlx 4	4 °C
Enzyme	Powder x 1	4 °C
Reaction Buffer	10 mlx 1	4 °C
Dye Reagent I	Powder x 2	4 °C
Dye Reagent II	Powder x 2	4 °C
Dye Reagent II Diluent	3 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use, store at 4 °C.

Dye Reagent I: Add 3.5 ml distilled water into 1 bottle Dye Reagent I to dissolve before use. Store at 4°C and use within 24 hours.

Dye Reagent II: Briefly centrifuge prior to opening. Add 1.5 ml Dye Reagent II Diluent into 1 vial Dye Reagent II, mix before use. Store at 4°C and use within 24 hours.

Standard: Briefly centrifuge prior to opening. 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. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

V. 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 5×10^6 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.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	70 µl	70 µl	70 µl
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Enzyme	10 µl	10 µl	10 µl
Dye Reagent I	70 µl	70 µl	70 µl
Dye Reagent II	30 µl	30 µl	30 µl
Mix, incubate at 37 °C for 5 min, record absorbance measured at 620 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** 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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

2.2 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times (W \times V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol/g})$$

2.3 According to the quantity of cells or bacteria

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times (N \times V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol}/10^4)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of sample, g

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

V_{Standard} : the volume of standard in assay procedure, mL

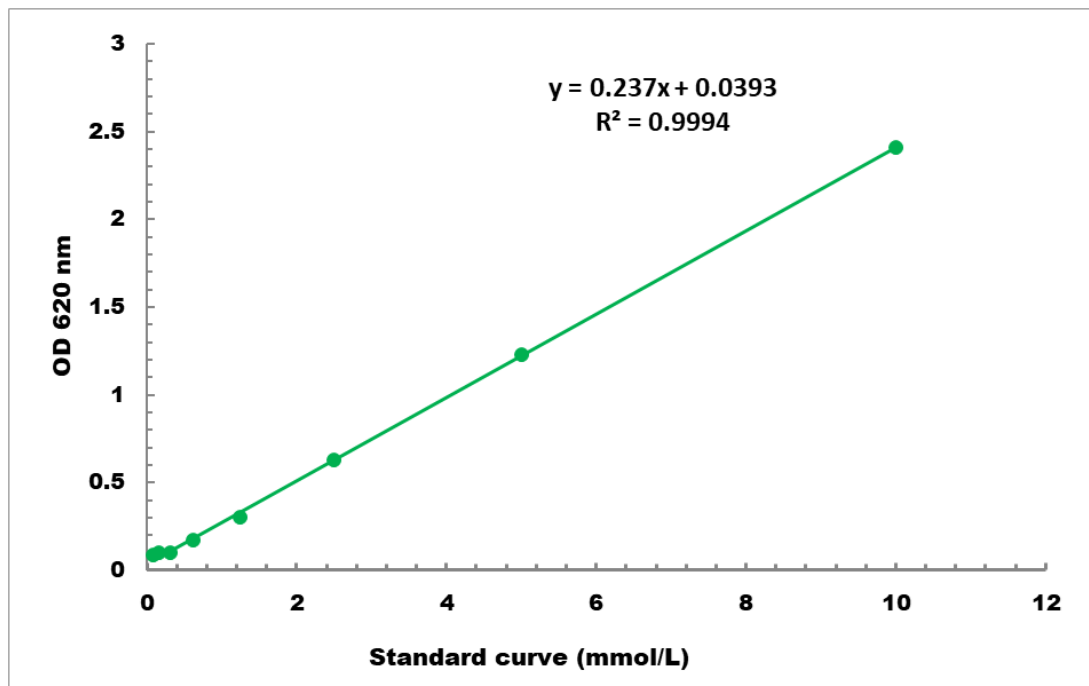
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, ml

C_{Standard} : the standard concentration, $\mu\text{mol/mL}$

VIII. 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