



Glutaminase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1065

(Version 2.4I)

Detection and Quantification of Glutaminase (GLS) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

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I. INTRODUCTION

Glutaminase (glutamine aminohydrolase or GLS) catalyzes the following reaction:

Glutamine + H₂O → Glutamate + NH₃. The enzyme has tissue-specific roles in multiple organs. Two different mammalian phosphate-activated GLS isoforms are known: GLS1 (kidney-type) and GLS2 (liver-type; a target of the tumor suppressor protein p53). The hydrolytic activity of glutaminase generates ammonia for urea synthesis in the liver similar to that mediated by glutamate dehydrogenase. During renal acidosis, glutaminase is induced in the kidney, leading to increased excretion of ammonia, which plays an important role in maintaining acid-base homeostasis.

Glutaminase regulates the levels of the neurotransmitter glutamate in the brain. The rate of glutaminolysis is known to increase in tumors and may be a hot spot for regulation of cancer cell metabolism. Inhibitors of GLS may therefore be candidate drugs for cancer therapy.

Glutaminase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining glutaminase activity in various samples. The assay is initiated with the enzymatic hydrolysis of glutamine by GLS. The enzyme catalysed reaction products can be measured at a colorimetric readout at 620 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Substrate	Powderx 1	4 °C
Stop Solution	30 mlx 1	RT
Reaction Buffer	2 mlx 1	RT
Dye Reagent I	Powder x 2	4 °C
Dye Reagent II	Powder x 2	4 °C
Dye Reagent IIDiluent	1 ml x 1	4 °C
Standard (1mmol/L)	1 mlx 1	4 °C
Positive Control	Powder x 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Dye Reagent I: Add 2 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 0.5 ml Dye Reagent II Diluent into 1 vial Dye Reagent II, mix before use. Store at 4 °C and use within 24 hours.

Substrate: Add 20 ml distilled water to dissolve before use, store at 4 °C. Store at -20 °C. Use within one month.

Positive Control: Briefly centrifuge prior to opening. Add 0.1 ml assay buffer to dissolve before use. Aliquot & store at -80 °C. Use within one month.

Standard: 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 1/0.5/0.25/0.125/0.0625/0.0312/0.0156 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 samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank	Positive Control
Sample	20 μ l	--	--	--	--
Distilled water	--	20 μ l	--	--	--
Positive Control	--	--	--	--	20 μ l
Substrate	200 μ l	200 μ l	--	--	200 μ l
Mix, put it in the oven, 37°C for 10 mins.					
Stop Solution	300 μ l	300 μ l	--	--	300 μ l
Mix, centrifuged at 10,000g for 5 minutes, add the supernatant into the microplate.					
Supernatant	130 μ l	130 μ l	--	--	130 μ l
Standard	--	--	130 μ l	--	--
Distilled water	--	--	--	130 μ l	--
Reaction Buffer	20 μ l	20 μ l	20 μ l	20 μ l	20 μ l
Dye Reagent I	40 μ l	40 μ l	40 μ l	40 μ l	40 μ l
Dye Reagent II	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
Mix, put it into the oven, 70°C for 5 minutes. Then 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

Unit Definition: one unit is defined as the enzyme will generate 1 μ mole ammonia per minute.

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

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times 4(\text{U/mL})$$

Calculate the initial activity according to sample preparation procedure.

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

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} \times 4(\text{U/mg/mL})$$

2.2 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times (W \times V_{\text{Sample}} / V_{\text{Assay}}) \times T} \times 4(\text{U/g})$$

2.3 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times (N \times V_{\text{Sample}} / V_{\text{Assay}}) \times T} \times 4(\text{U}/10^4)$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} \times 4(\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein} : the protein concentration, mg/mL

C_{Standard} : the concentration of standard, μ mol/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

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

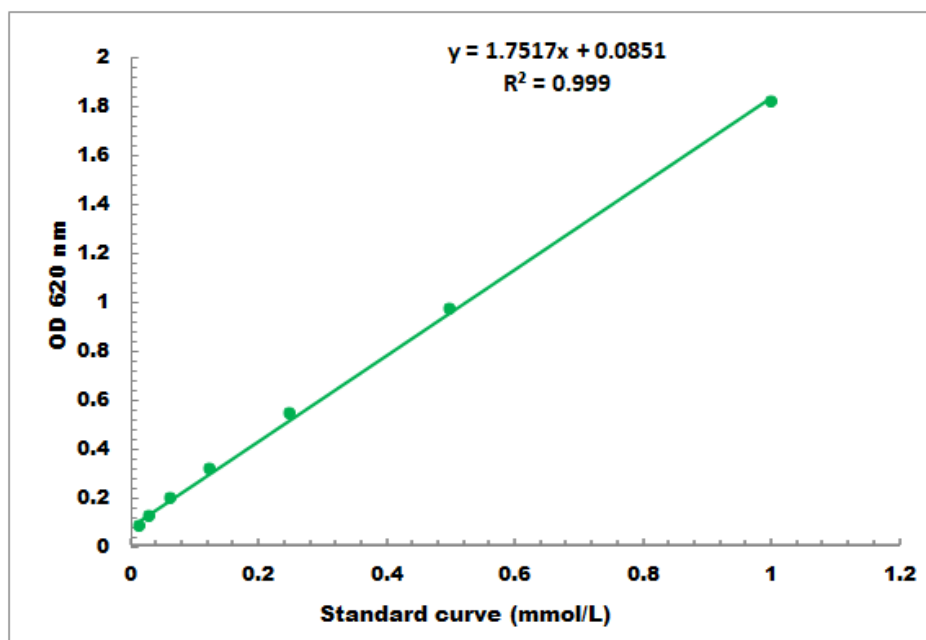
V_{Sample} : the volume of sample in assay procedure, mL

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

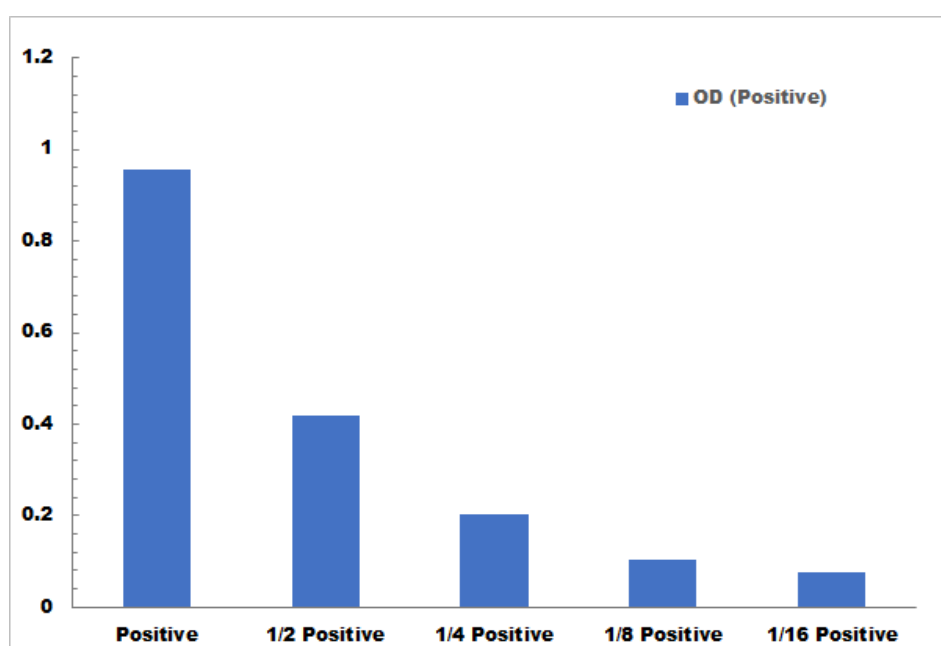
T: the reaction time, minute

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



Positive Control reaction in 96-well plate assay with decreasing the concentration