Glutaminase Microplate Assay Kit

User Manual

Catalog # CAK1065

(Version 2.3H)

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.

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 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	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Stop Solution	30 ml x 1	RT
Reaction Buffer	2 ml x 1	RT
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent II Diluent	1 ml x 1	4 °C
Standard (1 mmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	4 °C
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Note:

Dye Reagent I: add 4 ml distilled water to dissolve before use. Store at -20 °C. Use within one month.

Dye Reagent II: add 1 ml Dye Reagent II Diluent to dissolve before use. Store at -20 °C. Use within one month.

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

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

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. 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.

V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank	Positive Control
Sample	20 µl	--	--	--	--
Distilled water	--	20 µl	--	--	--
Positive Control	--	--	--	--	20 µl
Substrate	200 µl	200 µl	--	--	200 µl
Mix, put it in the oven, 37 °C for 10 mins.					
Stop Solution	300 µl	300 µl	--	--	300 µl
Mix, centrifuged at 10,000g for 5 minutes, add the supernatant into the microplate.					
Supernatant	130 µl	130 µl	--	--	130 µl
Standard	--	--	130 µl	--	--
Distilled water	--	--	--	130 µl	--
Reaction Buffer	20 µl	20 µl	20 µl	20 µl	20 µl
Dye Reagent I	40 µl	40 µl	40 µl	40 µl	40 µl
Dye Reagent II	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, put it into the oven, 70 °C for 5 minutes. Then record absorbance measured at 620 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time in first step; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time in first step.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: one unit is defined as the enzyme that generates 1 μmol of ammonia per minute.

1. According to the protein concentration of sample

$$GLS\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / C_{Protein} / T \times 4$$

$$= 2.6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$GLS\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T \times 4$$

$$= 2.6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$GLS\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T \times 4$$

$$= 2.6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$GLS\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T \times 4$$

$$= 2.6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

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

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

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

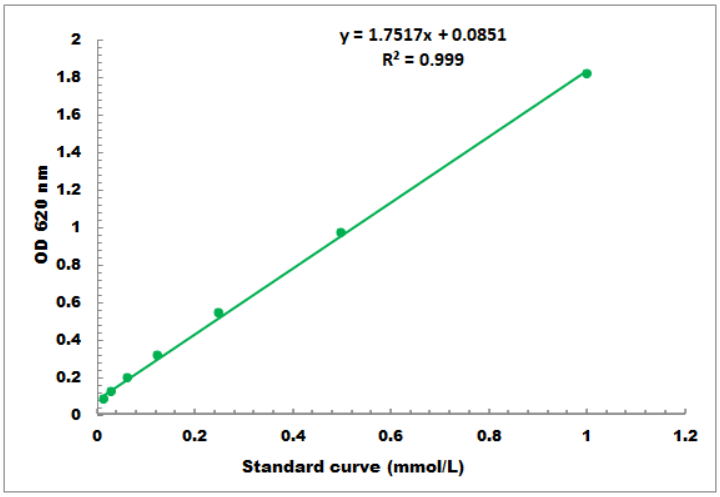
$V_{Standard}$: the volume of sample, 0.13 ml;

V_{Assay}: the volume of Assay buffer in sample preparation, 1 ml;

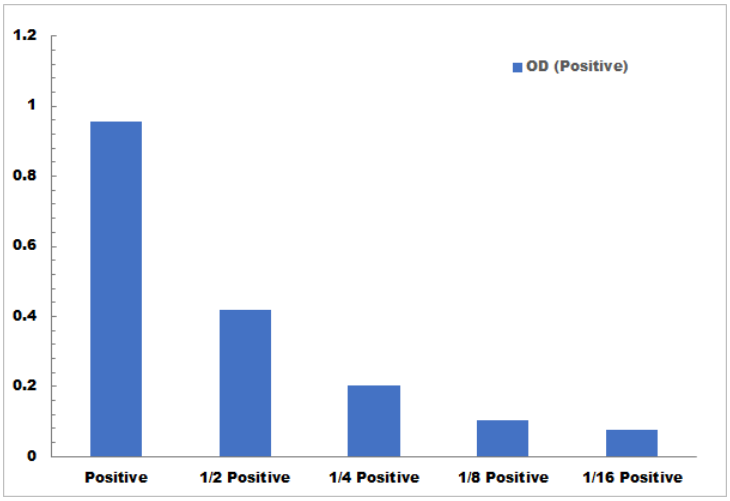
T: the reaction time, 10 mins.

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

VIII. TECHNICAL SUPPORT

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

IX. NOTES