Glutaminase Microplate Assay Kit

Catalog # AS0065

Detection and Quantification of Glutaminase Activity in Tissue extracts, Cell lysate Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic 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.

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 420 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	5 mlx 1	RT
Dye Reagent	2 mlx 1	RT
Standard (1μmol/ml)	1 mlx 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate: add 20 ml distilled water to dissolve before use, store at 4 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 420 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 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 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.

V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Sample	20 µl	--	--	--
Distilled water	--	20 µl	--	--
Substrate	200 µl	200 µl	--	--
Mix, put it in the oven,37°Cfor 1 hour.				
Stop Solution	300 µl	300 µl	--	--
Mix, centrifuged at 8,000g for 5minutes, add the supernatant intothe microplate.				
Supernatant	130 µl	130 µl	--	--
Standard	--	--	130 µl	--
Distilled water	--	--	--	130 µl
Reaction Buffer	50 µl	50 µl	50 µl	50 µl
Dye Reagent	20 µl	20 µl	20 µl	20 µl
Mix,record absorbance measured at 420 nm immediately.				

VI. CALCULATION

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

1. According to the protein concentration of sample

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

$= 4 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

2. According to the weight of sample

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

$= 200 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$

3.According to the quantity of cells or bacteria

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

$= 200 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$

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

$C_{Standard}$: the concentration of Standard, 1μmol/ml;

W: the weight of sample, g;

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

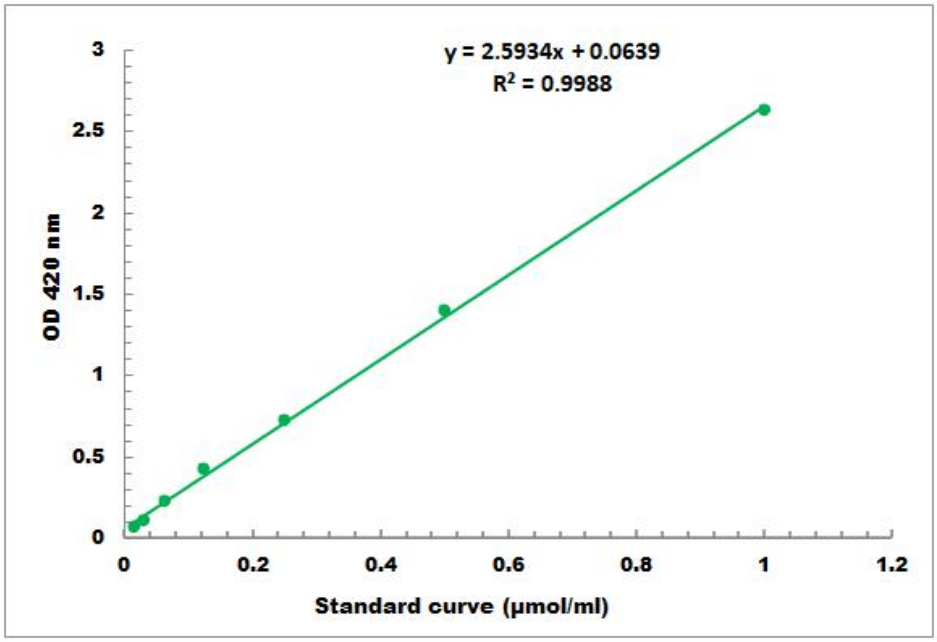
V_{Sample}: the volume of sample, 0.02 ml;

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

T: the reaction time, 1 hour.

VII. TYPICAL DATA

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



Detection Range: 10μmol/L -1μmol/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES