Glutaminase Assay Kit (Colorimetric)

Glutaminase Assay Kit (Colorimetric) is a detection kit for the quantification of Glutaminase Activity in cells, bacteria and tissue samples.

Catalog number: ARG82767

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

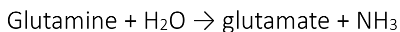
Email: info@arigobio.com

INTRODUCTION

GLS gene encodes the K-type mitochondrial glutaminase. The encoded protein is a phosphate-activated amidohydrolase that catalyzes the hydrolysis of glutamine to glutamate and ammonia. This protein is primarily expressed in the brain and kidney plays an essential role in generating energy for metabolism, synthesizing the brain neurotransmitter glutamate and maintaining acid-base balance in the kidney. Alternate splicing results in multiple transcript variants. [provided by RefSeq, Jan 2012]

Glutaminase catalyzes the first reaction in the primary pathway for the renal catabolism of glutamine. Plays a role in maintaining acid-base homeostasis. Regulates the levels of the neurotransmitter glutamate, the main excitatory neurotransmitter in the brain (PubMed:30575854, PubMed:30239721, PubMed:30970188). [UniProt]

Glutaminase catalyzes the following reaction:



Glutamine + H_2O → glutamate + NH_3

PRINCIPLE OF THE ASSAY

The Glutaminase Assay Kit is initiated with the enzymatic hydrolysis of glutamine by GLS. The enzyme catalyzed reaction products can be measured at a colorimetric readout at 620 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Microplate	1 X 96-well plate	4°C
Standard (1 µmol/ml)	1 ml	4°C
Positive Control	1 µl	4°C
Assay Buffer	4 X 30 ml (ready to use)	4°C
Reaction Buffer	2 ml	4°C
Substrate	1 vial (lyophilized)	4°C
Reaction Dye I	1 vial (lyophilized)	4°C
Reaction Dye II	1.5 ml	4°C
Stop Solution	30 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 620 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Convection oven (37°C)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Reaction Dye should be store at 4°C and protect from light.
- Briefly spin down the reagents before use.

- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell or bacteria lysate- Collect cell or bacteria in a centrifuge tube, wash cells 1-2X by PBS. Discard the supernatant after centrifugation, add 1 ml of Assay buffer pre 5×10^6 cell or bacteria in the tube. And then sonicate samples (set with power 20%, sonicate for 3 sec. and interval for 10 sec., repeat 30 times). Centrifuge samples 8000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Tissue lysate- Weigh out 0.1 g of tissue, homogenize with 1 ml Assay buffer on ice. Centrifuge samples 8000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Standard:** Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Reaction Dye I**: Reconstitute the Substrate with **3.5 ml** of **distilled water**. Allow the Reaction Dye I keep on bench for few minutes. Make sure the Reaction Dye I is dissolved completely and mixed thoroughly before use. Keep the reconstituted the Substrate on ice before use.
- **Substrate:** Reconstitute the Substrate with **20 ml** of **distilled water**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use. Keep the reconstituted the Substrate on ice before use. The reconstituted Substrate can be stored at 4°C for up to 1 week.
- **Positive Control:** Dilute the Positive Control with **100 µl** of **distilled water.** Make sure the Positive Control is mixed thoroughly before use. Keep the Positive Control on ice before use. The diluted Positive Control can be stored at 4°C for up to 1 week.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with **1X Assay buffer** before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample tube: Add **20 µl** per **samples** into each microcentrifuge tube.
2. Control tube: Add **20 µl** of **distilled water** into another microcentrifuge tube.
3. Positive Control tube: Add **20 µl** of **Positive Control** into another microcentrifuge tube.
4. Add **200 µl** of **Substrate** per tube into all Sample, Control and Positive Control tubes.
5. Mix well and incubate all Samples, Control and Positive Control tubes at **37°C** oven for **10 min**.
6. Add **300 µl** of **Stop solution** into each all sample, Control and Positive Control tubes.
7. Mix well and centrifuge the tubes at **10,000 x g for 5 minutes**, transfer **130 µl** of the supernatant into well of the 96-well microplate.
8. Standard well: Add **130 µl** of **standard** into standard well of the 96-well microplate.
9. Blank well: Add **130 µl** of **distilled water** into blank well of the 96-well microplate.
10. Add **20 µl** of **Reaction Buffer** per well into all wells including Sample, Control, Standard, Positive Control and Blank wells.
11. Add **35 µl** of **Reaction Dye I** per well into all wells.
12. Add **15 µl** of **Reaction Dye II** per well into all wells.
13. Mix well. Incubate at **37°C** oven for **15 min**.
14. Read the OD with a microplate reader at **620 nm**.

Summary of Glutaminase Assay Procedure

Reagent	Sample	Control	Positive Control	Standard	Blank
Sample	20 µl	-	-	-	-
Distilled water	-	20 µl	-	-	-
Positive control	-	-	20 µl	-	-
Substrate	200 µl	200 µl	200 µl	-	-
Mix well. Incubate all Sample tubes at **37°C** oven for **10 min**.					
Stop Solution	300 µl	300 µl	300 µl	-	-
Mix well and centrifuge the tubes at **10,000 x g for 5 minutes**.					
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
Reaction Dye I	35 µl	35 µl	35 µl	35 µl	35 µl
Reaction Dye II	15 µl	15 µl	15 µl	15 µl	15 µl
Mix well. Incubate at **37°C** oven for **15 min**.					
Read the OD with a microplate reader at **620 nm**.					

CALCULATION OF RESULTS

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

2. Calculate the average absorbance values for each set of samples, standard, positive control, control and blank.

3. Calculation:

A. Definition:

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

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

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 20 µl = 0.02 ml;

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

$V_{standard}$: the volume of standard sample, 130 µl = 0.13 ml;

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

T: the reaction time, 10 minutes.

4: Sample diluted by Substrate and Stop solution

(20+200+300)/130 = 4

B. Formula:

a). According to the protein concentration of sample

Glutaminase (U/mg) =

$4 \times [(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times C_{Protein} \times V_{Sample} \times T]$

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

b). According to the weight of sample

Glutaminase activity (U/g) =

$4 \times [(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{total}) \times T]$

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

c). According to the quantity of cells or bacteria

Glutaminase activity ($U/10^4$) =

$4 \times [(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{total}) \times T]$

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

d). According to the volume of sample

Glutaminase activity (U/ml) =

$4 \times [(C_{Standard} \times V_{Standard}) \times (OD_{Sample}- OD_{Control}) / [(OD_{Standard}- OD_{Blank}) \times V_{Sample} \times T]$

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

4. Detection range:

The detection range is from 0.01 µmol/ml – 1 µmol/ml.

5. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.