Glutamine Synthetase Activity Assay Kit (Colorimetric)

Glutamine Synthetase Activity Assay Kit (Colorimetric) is a detection kit for the quantification of Glutamine Synthetase Activity in tissue extract and cell lysate.

Catalog number: ARG82778

Package: 96 wells

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INTRODUCTION

Glutamine synthetase (GS) (EC 6.3.1.2) is an enzyme that plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine:

Glutamate + ATP + NH_3 → Glutamine + ADP + phosphate

Glutamine synthetase uses ammonia produced by nitrate reduction, amino acid degradation, and photorespiration. The amide group of glutamate is a nitrogen source for the synthesis of glutamine pathway metabolites.

Other reactions may take place via GS. Competition between ammonium ion and water, their binding affinities, and the concentration of ammonium ion, influences glutamine synthesis and glutamine hydrolysis. Glutamine is formed if an ammonium ion attacks the acyl-phosphate intermediate, while glutamate is remade if water attacks the intermediate. Ammonium ion binds more strongly than water to GS due to electrostatic forces between a cation and a negatively charged pocket. Another possible reaction is upon NH_2OH binding to GS, rather than NH_4+, yields γ-glutamylhydroxamate. [Provide by Wikipedia: Glutamine synthetase]

PRINCIPLE OF THE ASSAY

This Glutamine Synthetase Activity Assay Kit is a simple colorimetric assay that measures the activity of Glutamin Synthetase (GS) in tissue extract and cell lysate sample. The assay is based on the enzyme driven reaction. In the presence of ATP and Mg^{2+}, GS can catalyze ammonium ions and glutamic acid to synthesise glutamine Gln, and the glutamine Gln further converted to gamma-glutamyl hydroxamic acid, under acidic conditions to form a red iron complexes. The complex has a maximum absorption peak at O.D. 540 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store substrate at -20°C. Store the other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
96 Well Microplate	1 plate	RT
Assay Buffer	4 x 30 mL (ready to use)	4°C
Standards (lyophilized)	1 vial	4°C
Substrate (lyophilized)	1 vial	-20°C
Dye Reagent	5 mL (ready to use)	4°C
Reaction Buffer	8 mL (ready to use)	4°C
Plate Sealer	3 strips	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 540 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Convection oven (37°C)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store substrate at -20°C. Store the other component at 2-8°C.
- 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; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in 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.

Tissue lysate: Weigh out 0.1 g tissue, homogenize with 1 mL of Assay Buffer on ice, centrifuged at 8,000 x g for 10 minutes at 4°C. 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.

Cell lysate: Collect cell into centrifuge tube, discard the supernatant after centrifugation, add 1 mL of Assay Buffer for 5×10^6 cell, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8,000 x g for 10 minutes at 4°C. 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.

For other Liquid samples: Detect directly.

REAGENT PREPARATION

- Substrate: Reconstitute the Substrate with 3.5 ml of distilled water. 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 a week.
- Standard: Reconstitute the Standard with 1 ml of distilled water. Make sure the Standard is dissolved completely and mixed thoroughly before use. Keep the reconstituted standard stock at 4°C. Before assay, add 0.25 mL of standard stock into 0.75 mL of distilled water to yield a working standard concentration of 5 mmol/L.

ASSAY PROCEDURE

Standard and samples should be assayed in duplicate or triplicate.

1. Add **80 µL** of **Reaction Buffer** into sample and control wells of the plate.
2. Add **35 µL** of **Substrate** into sample and control wells of the plate.
3. Add **35 µL** of **Distilled water** into control wells.
4. Add **35 µL** of **each Sample** into sample wells.
5. Mix well. Cover the plate with Plate Sealer and incubate the plate for **30 minutes at 37°C** in the oven.
6. Add **150 µL** of **Standard** into Standard wells.
7. Add **150 µL** of **Distilled water** into Blank wells.
8. Add **50 µL** of **Dye Reagent** into **all** wells.

Summary of Glutamine Synthetase Activity Assay Procedure

	Sample	Control	Standard	Blank
Reaction Buffer	80 µL	80 µL	-	-
Substrate	35 µL	35 µL	-	-
Distilled water	-	35 µL	-	-
Each Sample	35 µL	-	-	-
Mix well. Incubate for 30 minutes at 37°C in the oven.				
Standard	-	-	150 µL	-
Distilled water	-	-	-	150 µL
Dye Reagent	50 µL	50 µL	50 µL	50 µL
Mix well. Read the absorbance at O.D. 540 nm.				

Note:

- Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 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; if the enzyme activity is higher, please dilute the sample with 1X Assay buffer, or decrease the reaction time. For the calculation of the activity this dilution factor has to be taken into account.

CALCULATION OF RESULTS

1. Unit Definition: one unit is defined as the enzyme products 1 nmol of the gamma-glutamyl hydroxamic acid per minute.
2. Calculate the average absorbance value for each set of Standards, Blank and samples.
3. Calculation:

   A. Definition:

   $C_{Protein}$: the protein concentration of sample, mg/mL;

   W: the weight of sample, g;

   $C_{Standard}$: the concentration of standard, 5 mmol/L = 5000 nmol/mL;

   $V_{Standard}$: the volume of standard, 0.15 mL;

   V_{Sample}: the volume of sample, 0.035 mL;

   V_{Assay}: the volume of Assay buffer, 1 mL;

   T: the reaction time, 30 minutes.

   B. Formula:

   a). According to the protein concentration of sample

   Glutamine Synthetase Activity (U/mg)

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

   $= 714.3 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]$

   b). According to the weight of sample:

   Glutamine Synthetase Activity (U/g)

   $= [(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times W \times (1/V) \times T]$

   $= 714.3 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times W]$

EXAMPLE OF TYPICAL STANDARD CURVE

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 this kit does not need serial diluted standards.