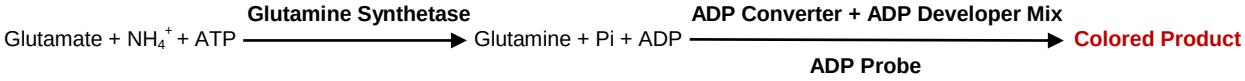
Glutamine Synthetase Activity Assay Kit (Colorimetric)

09/20

(Catalog # K2056-100; 100 assays; Store at -20°C)

I. Introduction:

Glutamine Synthetase (GS, Glutamate-Ammonia Ligase; EC 6.3.1.2) is a mitochondrial enzyme that catalyzes the ATP-dependent ligation of glutamate and ammonia to glutamine. GS is ubiquitously expressed in human tissues and plays a critical role in detoxifying ammonia and regulating the concentration of glutamate or glutamine. It is also a key enzyme in nitrogen metabolism and a principal source for protein and nucleic acid synthesis. Additionally, GS activity has been linked to several neurological disorders such as Alzheimer's disease, schizophrenia, hepatic encephalopathy and epilepsy. Furthermore, two mutations of human GS (R324C and R341C) have been linked to congenital glutamine deficiency with severe brain malformations resulting in multi-organ failure and neonatal death. **BioVision's Glutamine Synthetase Activity Assay Kit** is a simple, plate-based colorimetric assay for measuring GS activity in biological samples. In this assay, GS hydrolyzes Glutamate to Glutamine and ADP. ADP in the subsequent enzymatic reaction, in presence of ADP Converter, ADP Developer Mix and ADP Probe forms a colorimetric product that is measured at OD 570 nm. The assay is simple, sensitive and can detect as low as 10 µU of GS in biological samples.



II. Application:

- Measurement of Glutamine Synthetase activity in tissue and cell lysate

III. Sample Types:

- Tissue lysates, i.e. Brain tissue
- Cell Lysates, i.e. 3T3 cell lysates

IV. Kit Contents:

Components	K2056-100	Cap Code	Part Number
GS Assay Buffer	35 ml	NM	K2056-100-1
Glutamine Synthetase	1 vial	Blue	K2056-100-2
Glutamate	1.2 ml	White	K2056-100-3
ATP	2 vials	Orange	K2056-100-4
ADP Probe	0.2 ml	Red	K2056-100-5
ADP Converter	1 vial	Purple	K2056-100-6
ADP Developer Mix	1 vial	Green	K2056-100-7
ADP Standard	1 vial	Yellow	K2056-100-8

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer
- 96-well clear plate with flat bottom
- Deionized water
- 10 kDa Spin Column (BioVision Cat# 1997)

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protect from light. Briefly centrifuge all small vials prior to opening. Read entire protocol before performing the assay.

- **GS Assay Buffer:** Thaw to room temperature (RT) before use. Store at 4°C or -20°C, protected from light.
- **Glutamine Synthetase:** Reconstitute the vial in 100 µl GS Assay Buffer. Pipette up and down to mix well. Divide into aliquots and store at -20°C. Keep on ice while in use.
- **Glutamate:** Divide into aliquots and store at -20°C. Keep on ice while in use.
- **ATP:** Dissolve 1 vial of ATP with 55 µl **dH_2O** to prepare the ATP stock solution. Pipette up and down to dissolve completely. Each vial can be used to carry out up to 50 reactions. Dissolve vial contents when needed. Store at -20 °C. Keep on ice while in use. Use within two months.
- **ADP Probe (in DMSO):** Ready to use as supplied. Warm up to RT (to melt the frozen DMSO) before use. Mix well. Store at -20°C, protect from light and moisture. Use within two months.
- **ADP Converter** and **ADP Developer Mix:** Reconstitute each of the vials in 220 µl GS Assay Buffer separately. Pipette up and down to dissolve the contents. Store at -20°C. Keep on ice while in use. Use within two months.
- **ADP Standard:** Reconstitute the vial in 100 µl dH_2O to generate 10 mM ADP Standard stock solution. Keep on ice while in use. Store at -20°C.

VII. Glutamine Synthetase Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (~10 mg) or pelleted cells (~4 x 10^5) with 200 µl ice-cold GS Assay Buffer and keep on ice for 10 min. Centrifuge samples at 12,000 x *g* for 15 min at 4°C, collect the supernatant. Estimate the protein concentration using preferred method. We recommend BCA protein assay kit (BioVision Cat# K813-2500). Protein concentration should range between 0.1 and 1 µg/µl. Dilute samples if needed using GS Assay Buffer. For removal of small molecules that may interfere with the results, filter the samples through a 10 kDa Spin Column (BioVision Cat# 1997). Centrifuge at 12,000 x *g* and 4°C for 10 min and discard the filtrate. Adjust the ultra-concentrate to the original volume using GS Assay Buffer and repeat this procedures 3-5 times. The ultraconcentrate should be used for GS activity assay. For each sample type, add 2-20 µl of samples into two wells of a 96-well, clear plate labeled as **Sample** and **Sample Background Control.** Add 20 µl of GS Assay Buffer to a well labeled as **Reagent Background Control**. For

Positive Control, add 8-12 µl of reconstituted Glutamine Synthetase into the desired well(s). Adjust the volume to 20 µl/well with GS Assay Buffer.

Note: For Unknown Samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.

2. **Standard Curve Preparation:** Prepare 10-fold dilution of the 10 mM ADP Standard stock solution, to 1 nmol/µl by adding 6 µl of 10 mM (10 nmol/µl) ADP Standard stock to 54 µl of GS Assay Buffer and mix well. Add 0, 2, 4, 6, 8 10 µl of 1 nmol/µl ADP Standard into a series of wells to generate 0, 2, 4, 6, 8, 10 nmol/well ADP Standard respectively. Adjust the volume to 20 µl/well with GS Assay Buffer.
3. **Substrate Mix Preparation:** Prepare 10 fold dilution of ATP by mixing 10 µl of ATP stock solution with 90 µl GS Assay Buffer. Mix enough reagents for the number of assays to be performed. For each well, prepare 60 µl Reaction Mix and 60 µl Sample Background Mix containing:

	Reaction Mix	Sample Background Mix
GS Assay Buffer	40 µl	50 µl
Glutamate	10 µl	10 µl
Diluted ATP	10 µl	--

Mix well. Add 60 µl of Reaction Mix to ADP Standard(s), Sample(s) and Reagent Background Control wells. Add 60 µl of Sample Background Mix to Sample Background Control well(s). ***The total volume of each well is 80 µl.***

4. **ADP Reaction Mix:** Mix enough reagents for the number of assays to be performed: For each well, prepare 20 µl ADP Reaction Mix containing:

	ADP Reaction Mix
GS Assay Buffer	14 µl
ADP Probe	2 µl
ADP Converter	2 µl
ADP Developer	2 µl

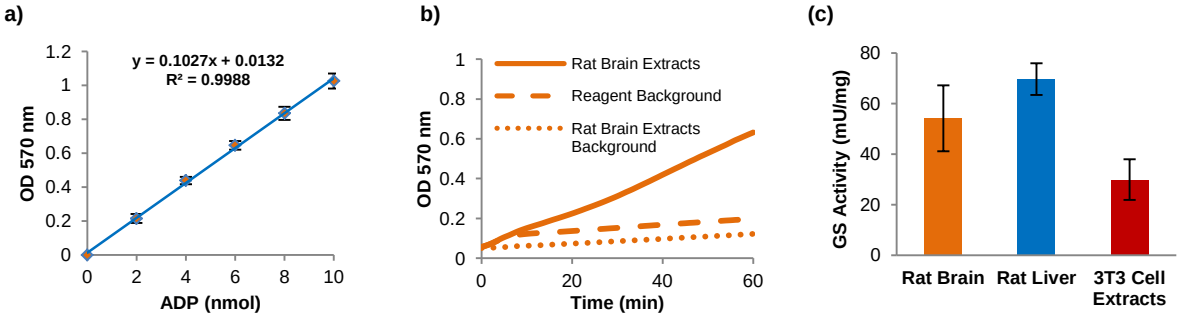
Mix well. Add 20 µl of ADP Reaction Mix to wells containing ADP Standard(s), Sample(s), Reagent Background Control and Sample Background Control. Mix well.

5. **Measurement:** Measure the absorbance immediately at 570 nm in kinetic mode for 60 min at 37°C. The ADP Standard(s) can be read in end point mode (t = 60 min) at 37°C, protected from light.
6. **Calculation:** Subtract the 0 Standard reading from all Standards readings and plot the ADP Standard Curve. Choose any two time points within the linear range of the curve (t_1 & t_2) for each sample type. Subtract the Sample Background Control reading from the corresponding Sample readings for the chosen t_1 & t_2 time points. **If the Reagent Background Control reading is higher than the Sample Background Control reading, subtract the Reagent Background Control readings from the Sample readings instead.** Apply the corrected Sample readings to the ADP Standard Curve to get **B** nmol of ADP generated during the reaction time ($\Delta t = t_2 - t_1$).

$$\text{Sample Glutamine Synthetase Activity} = \frac{(\text{B Sample} - \text{B Sample Background Control})}{\Delta t * V * P} * D = \text{nmol/min/mg} = \text{mUnits/mg}$$

Where: **B** = ADP amount from the ADP Standard Curve (nmol)
V = Sample volume added into the reaction well (ml)
P = Initial Sample Concentration in mg-protein/ml (mg/ml)
Δt = Reaction time (min)
D = Dilution factor (D = 1, for undiluted samples)

Unit Definition: One unit of Glutamine Synthetase activity was defined as the amount of Enzyme that produces 1 µmol of ADP per minute at pH 7.2 at 37°C.



Figures. a). ADP Standard Curve. **b)** Enzyme kinetics of rat brain extracts (1.3 µg). **c)** Glutamine Synthetase specific activity in rat brain (1.3 µg), rat liver (1.0 µg) and 3T3 cell lysates (1.0 µg). All assays were performed following kit protocols.

VIII. Related Products:

PicoProbe™ Glutamate Assay Kit (Fluorometric) (K413)
PicoProbe™ Glutaminase Activity Assay Kit (K455)
Glutamine Colorimetric Assay Kit (K556)
Asparagine Assay Kit (Fluorometric) (K736)
Glutaminase (GLS1) Inhibitor Screening Kit (Fluorometric) (K479)
Transglutaminase Inhibitor Screening Assay Kit (K508)
Glutamate Colorimetric Assay Kit (K629)
Glutamine Synthetase, human recombinant (P1067)

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