



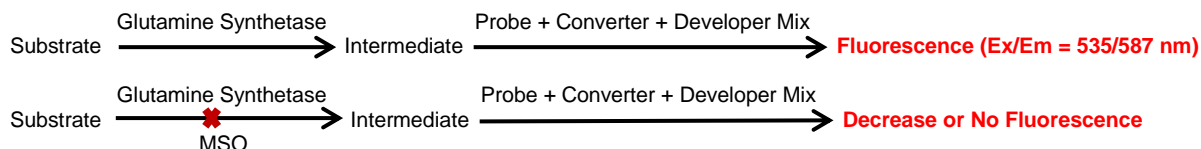
Glutamine Synthetase Inhibitor Screening Kit (Fluorometric)

10/20

(Catalog # K2059-100; 100 assays; Store at -20°C)

I. Introduction:

Glutamine Synthetase (GS; EC 6.3.1.2) is a key enzyme that regulates nitrogen metabolism by catalyzing the formation of glutamine from glutamate and ammonia. It is ubiquitously expressed in humans and is linked to several neurological disorders including Alzheimer's disease, schizophrenia, epilepsy etc. Inhibition of GS in animal models have shown significant therapeutic benefits in hepatic encephalopathy, amyotrophic lateral sclerosis, inflammatory liver failure, cancer and other human diseases. Additionally, GS is highly expressed in bacteria and is essential for its growth. Inhibition of *Mycobacterium tuberculosis* GS has been used as a therapeutic strategy against Tuberculosis. **BioVision's Glutamine Synthetase Inhibitor Screening Kit** is a plate-based fluorometric assay for the screening human GS inhibitors. In this kit, the activity of GS is monitored by the formation of a fluorescent product formed via an enzymatic reaction and is detected at Ex/Em = 535/587 nm. But in the presence of GS inhibitors, the GS enzyme loses its activity, which results in the decrease of fluorescence intensity. Methionine Sulfoximine (MSO), a well-characterized inhibitor of prokaryotic and eukaryotic GSs with different K_i values is used as the positive control. The assay is simple and can be used to identify and characterize human GS inhibitors.



II. Application:

- For screening potential inhibitors of Human Glutamine Synthetase

III. Kit Contents:

| Components | K2059-100 | Cap Code | Part Number |
|------------------------------|-----------|----------|-------------|
| GS Assay Buffer | 35 ml | NM | K2059-100-1 |
| Glutamate | 1.2 ml | White | K2059-100-2 |
| ATP | 1 vial | Orange | K2059-100-3 |
| GS Probe | 0.2 ml | Red | K2059-100-4 |
| GS Converter | 1 vial | Purple | K2059-100-5 |
| GS Developer Mix | 1 vial | Green | K2059-100-6 |
| Glutamine Synthetase | 1 vial | Blue | K2059-100-7 |
| Methionine Sulfoximine (MSO) | 110 µl | Amber | K2059-100-8 |

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- Distilled Water

V. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials before opening. Read the entire protocol before performing the assay. Components are stable for at least two months.

- **GS Assay Buffer:** Thaw at room temperature (RT) before use. Store at 4°C or -20°C, protected from light.
- **Glutamate:** Divide into aliquots and store at -20°C. Keep on ice, while in use.
- **ATP:** Reconstitute the vial in 55 µl dH₂O to prepare the ATP stock solution. Pipette up and down to dissolve completely. Store at -20°C. Keep on ice, while in use. Use within two months.
- **GS 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, protected from light and moisture. Use within two months.
- **GS Converter and GS 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, protected from light. Use within two months.
- **Glutamine Synthetase:** Reconstitute the vial in 110 µl GS Assay Buffer to prepare the stock GS enzyme. Pipette up and down to mix well. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice, while in use.
- **Methionine Sulfoximine (MSO):** Ready to use. Divide into aliquots and store at -20°C. Keep on ice, while in use.

VI. Glutamine Synthetase Inhibitor Screening Protocol:

1. Test Compound, Inhibitor Control, Enzyme Control and Background Control Preparations: Test Compound [S]: Dissolve the Test Compound(s) in appropriate solvent. Further dilute to proper concentration using GS Assay Buffer. Add 10 µl of diluted Test Compound(s) into each well of a 96-well clear plate. Prepare a parallel well labeled as Solvent Control [SC] with the same final concentration of solvent used to solubilize the Test Compound(s). For Enzyme Control (No inhibitor) [EC] and Background Control [BC], add 10 µl of GS Assay Buffer to the each well. For Inhibitor Control [IC], add 10 µl of Methionine Sulfoximine into the designated wells.



Notes:

- a). Organic solvents used to prepare the Test Compound(s) stock may impact GS activity. To determine the effect of the solvent on GS activity, we recommend preparing parallel Solvent Control wells with the same final concentration of solvent used to solubilize the Test Compounds(s).
- b). We recommend testing several dilutions of the Test Compound with each well containing 10 µl of Test Compound.
2. **GS Enzyme Working Solution Preparation:** Prepare a 10-fold dilution of the stock GS Enzyme by adding 10 µl of stock GS Enzyme to 90 µl of GS Assay Buffer. Mix thoroughly and keep on ice. Mix enough reagents for the number of assays to be performed. For each well, prepare 30 µl Mix containing:

| | <u>Enzyme Mix</u> | <u>Background Mix</u> |
|-------------------|-------------------|-----------------------|
| GS Assay Buffer | 20 µl | 30 µl |
| Diluted GS Enzyme | 10 µl | - |

Mix well and add 30 µl Enzyme Mix to Test Compound [S], Inhibitor Control [IC], Enzyme Control [EC] and Solvent Control [SC] wells and add 30 µl Background Mix to Background Control [BC] well. Mix well. **Incubate the plate at 37°C for 5-10 min, protected from light.**

3. **Substrate Mix Preparation:** Prepare a 10-fold dilution of Glutamate by adding 10 µl of Glutamate stock solution to 90 µl GS Assay Buffer and mix well. Prepare a 10-fold dilution of ATP by adding 10 µl of ATP stock solution to 90 µl GS Assay Buffer and mix well. Mix enough reagents for the number of assays to be performed. For each well, prepare 40 µl Substrate Mix containing:

| | <u>Substrate Mix</u> |
|-------------------|----------------------|
| GS Assay Buffer | 30 µl |
| Diluted Glutamate | 6 µl |
| Diluted ATP | 4 µl |

Mix and add 40 µl of Substrate Mix to all wells including [S], [IC], [EC], [SC] and [BC] and mix well.

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

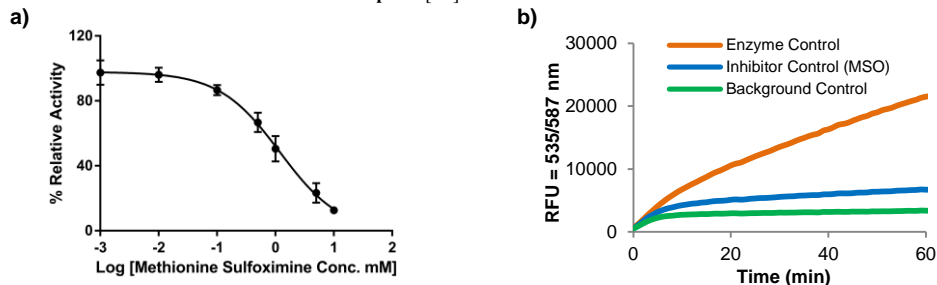
| | <u>Reaction Mix</u> |
|-----------------|---------------------|
| GS Assay Buffer | 17 µl |
| GS Probe | 1 µl |
| GS Converter | 1 µl |
| GS Developer | 1 µl |

Mix and add 20 µl Reaction Mix to all wells including [S], [IC], [EC], [SC] and [BC] and mix well. **The total reaction volume in each well is 100 µl.**

5. **Measurement:** Read fluorescence immediately in kinetic mode at Ex/Em = 535/587 nm for 60 min at 37°C. Choose any two time points (t₁ & t₂) in the linear range of the assay and obtain the corresponding fluorescence values (RFU₁ and RFU₂).
6. **Calculation:** Calculate the slope for [S], [EC], [SC] and [BC] by dividing the ΔRFU (RFU₂ - RFU₁) over reaction time Δt (t₂-t₁). Subtract the Slope of [BC] from [S], [EC] and [SC]. If [SC] slope is significantly different when compared to [EC], use [SC] values to determine the effect of Test Compound.

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of [EC]} - \text{Slope of [S]}}{\text{Slope of [EC]}} \times 100$$

$$\% \text{ Relative Activity} = \frac{\text{Slope of [S]}}{\text{Slope of [EC]}} \times 100$$



Figures: a). Inhibition of Human Glutamine Synthetase activity by MSO. IC₅₀ of MSO was determined to be 1.1 ± 0.1 mM. b). Enzyme kinetics in the presence and absence of MSO. Assays were performed using kit protocol.

VII. RELATED PRODUCTS:

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|--|---|
| Glutamine Synthetase Activity Assay Kit (K2056) | Glutamine Synthetase, human recombinant (P1067) |
| PicoProbe™ Glutamate Assay Kit (Fluorometric) (K413) | Glutaminase (GLS1) Inhibitor Screening Kit (K479) |
| PicoProbe™ Glutaminase Activity Assay Kit (K455) | Transglutaminase Inhibitor Screening Assay Kit (K508) |

FOR RESEARCH USE ONLY! Not to be used on humans.