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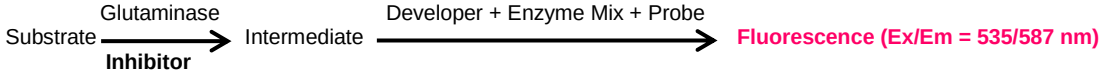
Glutaminase (GLS1) Inhibitor Screening Kit (Fluorometric)

rev 05/20

(Catalog # K479-100; 100 assays; Store at -20ºC)

I. Introduction:

Glutaminase (EC 3.5.1.2, GLS) is an enzyme that hydrolyzes glutamine producing glutamate and ammonia. It has tissue-specific roles in multiple organs, some of which include ammonia generation for urea synthesis in the liver; maintenance of acid-base homeostasis by ammonia production during renal acidosis in the kidney; and regulation of neurotransmitter glutamate in the brain. The isoform glutaminase 1 (GLS1) or phosphate-activated mitochondrial glutaminase is critical for glutamine utilization by cancer cells and the rate of glutaminolysis is known to increase in tumors, making it extremely important for tumor cell metabolism. GLS1 inhibitors have been considered as potential candidate drugs for cancer therapy. BioVision's Glutaminase (GLS1) Inhibitor Screening Kit is a plate-based fluorometric kit for screening human GLS1 inhibitors. The hydrolysis of glutamine by glutaminase forms glutamate and ammonia. Glutamate, in the presence of a developer and enzyme mix, converts a non-fluorescent probe to a fluorescent product via an enzymatic reaction**.** A well-known Glutaminase inhibitor - CB-839 is provided as an inhibitor control.



II. Applications:

- Screening of potential inhibitors of Glutaminase

III. Kit Contents:

Components	K479-100	Cap Code	Part Number
GLS Assay Buffer	25 ml	WM	K479-100-1
GLS Dilution Buffer	200 µl	Amber	K479-100-2
GLS Substrate	1 vial	White	K479-100-3
GLS Developer	1 vial	Green	K479-100-4
GLS Enzyme Mix	1 vial	Red	K479-100-5
GLS Probe	0.4 ml	Blue	K479-100-6
GLS1	20 µl	Purple	K479-100-7
GLS Inhibitor (CB-839)	20 µl	Orange	K479-100-8

IV. User Supplied Reagents and Equipment:

- 96-well white 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 entire protocol before performing the assay. Components are stable for at least two months.

- **GLS Buffer:** Warm to room temperature (RT) before use.
- **GLS Dilution Buffer:** Keep on ice when in use.
- **GLS Substrate**: Reconstitute in 220 µl water. Heat on a water bath at 37 ºC for 15 min to allow it to dissolve completely. Aliquot and store at -20ºC
- **GLS Probe and Inhibitor:** Thaw GLS probe and inhibitor at RT before use. Aliquot and store at -20ºC in the dark.
- **GLS Developer and Enzyme Mix:** Reconstitute each vial with 220 µl GLS Buffer. Aliquot and store at -20ºC.
- **GLS:** Always keep on ice. Aliquot into four aliquots of 5 µl each and store at -20ºC. **Prior to use,** dilute one aliquot at a time 10-fold in the provided **GLS dilution buffer** and mix by pipetting very gently. ***DO NOT VORTEX. DO NOT DILUTE IN GLS ASSAY BUFFER.***

  Note: *Keep GLS, GLS Developer and GLS Enzyme mix on ice while performing the assay.*

VI. Glutaminase Inhibitor Screening Assay Protocol:

1. **Test Compound Preparation:**
   Test Compounds [S]: Dissolve the test compound in appropriate solvent. Prepare at such concentration so volume of test compound solution added to a well is no more than 2 µl in the final 100 µl reaction volume per well (5% V/V). Add 2 µl test compound to each well of the 96-well white plate.
   Solvent Control [SC]: add 2 µl of the solvent used to prepare test compound solution at its final concentration in test wells.
   Inhibitor Control [IC]: add 2 µl of CB-839, the provided Glutaminase inhibitor.
   Bring up the volume of [S], [SC] and [IC] to 50 µl with GLS Assay Buffer.
   Enzyme Control [EC] and Substrate Background Control [BC]: add 50 µl GLS Assay Buffer to a well.
2. **Reaction Mix: Dilute one aliquot of glutaminase as described above in Glutaminase Dilution Buffer.** Mix enough reagents for the number of assays to be performed. Add Reaction Mix to [S], [SC], [IC] and [EC]. Add Background Mix to [BC]. For each well, prepare 20 µl Mix containing:

	Reaction Mix	Background Mix
Glutaminase Assay Buffer	18 µl	20 µl
Diluted Glutaminase	2 µl	-

   Incubate at RT for 10 minutes.

3. Substrate Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 30 µl Mix containing

	Substrate Mix
GLS Assay Buffer	22 µl
GLS Substrate	2 µl
GLS Developer	2 µl
GLS Enzyme Mix	2 µl
GLS Probe	2 µl

Mix well and add 30 µl of the Substrate Mix to all wells.

Note: have the plate reader ready at Ex/Em = 535/587 nm on kinetic mode set to record fluorescence every 30 sec at 37°C.

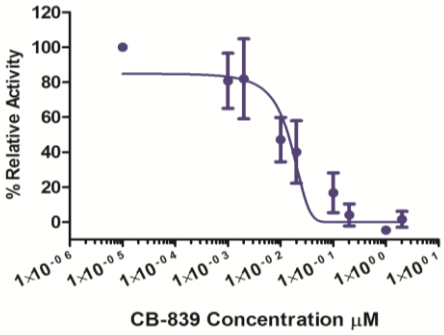
4. Measurement: Start recording fluorescence at Ex/Em = 535/587 nm after adding the substrate at 30 second intervals for 30 min.

5. Calculation: Subtract "Substrate Background Control" RFU values from RFU values for all other groups to obtain background subtracted RFU values. Obtain Δ RFU for all test compounds, enzyme control, solvent control and inhibitor control by subtracting RFU at time t_1 from RFU at time t_2, such that t_2 and t_1 is within a linear range of the assay. Calculate slope for all samples, including "enzyme control" by dividing ΔRFU by time Δt ($t_2 - t_1$). If "Solvent Control" slope is significantly different from "Enzyme Control" slope, use its values instead of "Enzyme Control" in the calculations shown below.

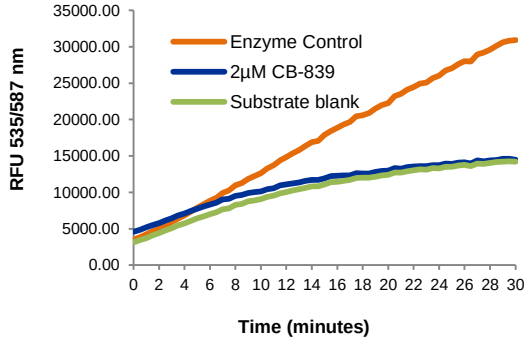
$$\% \text{ Inhibition} = \frac{[slope\ of\ (enzyme\ control) - slope\ of\ (test\ compound)]}{slope\ of\ (enzyme\ control)}\ X\ 100$$

$$\% \text{ Relative activity} = \frac{[slope\ (test\ compound)]}{slope\ (enzyme\ control)}\ X\ 100$$

a)



b)



Figures: a. Inhibition of Glutaminase activity by CB-839. IC_{50} of CB-839 was determined to be 13 ± 2.8 nM. **b**. Enzyme kinetics in presence and absence of CB-839. Assays were performed using kit protocol.

VII. RELATED PRODUCTS:

CB-839 (B1179)
Transglutaminase Activity Assay Kit (Colorimetric) (K571)
Transglutaminase Inhibitor Screening Assay Kit (K508)

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