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**Data Sheet**  
***GLS1 Inhibitor Screening Assay Kit***  
**Catalog #79596**  
**Size: 96 reactions**

**BACKGROUND:** Kidney-type glutaminase (GLS1) is a phosphate-activated amidohydrolase that catalyzes the hydrolysis of L-glutamine to L-glutamate and ammonia. GLS1 is primarily expressed in the brain and kidney where it catalyzes the first reaction in the primary pathway for the renal catabolism of glutamine. It plays an essential role in generating energy for metabolism, synthesizing the brain neurotransmitter glutamate and maintaining acid-base balance in the kidney.

**DESCRIPTION:** The *GLS1 Inhibitor Screening Assay Kit* is designed to measure the hydrolase activity of GLS1 for screening and profiling applications. The GLS1 assay kit comes in a convenient 96-well format, with purified GLS1, its substrates, the Coupling reagent, and GLS1 buffer for 100 enzyme reactions. In addition, the kit includes the GLS1 inhibitor CB-839 for use as a control inhibitor.

**COMPONENTS:**

Catalog #	Component	Amount	Storage	
71102	GLS1, His-tag*	1 µg	-80 °C	Avoid multiple freeze/thaw cycles!
	L-Glutamine (100 mM)	100 µl	-20 °C	
	NAD <sup>+</sup> (20 mM)	1 ml	-20 °C	
	Coupling reagent	10 µl	-20 °C	
79948	4X GLS assay buffer**	2.5 ml	-20 °C	
	CB-839 (100 µM)	20 µl	-20 °C	
79685	96-well black microplate	1	Room Temp.	

\* The concentration of GLS1 is lot-specific and will be indicated on the tube containing the enzyme.

\*\* Add 20 µl of 0.5 M DTT to 2.5 ml 4X GLS1 assay buffer before use

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

0.5 M DTT in aqueous solution

Adjustable micropipettor and sterile tips

Fluorescent microplate reader capable of reading  $\lambda_{excitation} = 340 \text{ nm}$ ;  $\lambda_{emission} = 460 \text{ nm}$

**APPLICATIONS:** Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

**STABILITY:** Up to 6 months from date of receipt, when stored as recommended.

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#### REFERENCES:

1. Mates, J. M., *et al.* (2013). Glutaminase isoenzymes as key regulators in metabolic and oxidative stress against cancer. *Current Molecular Medicine* **13(4)**: 514-534.
2. Gross, Matthew I., *et al.* (2014). Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. *Molecular Cancer Therapeutics* **13(4)**: 890-901.

#### ASSAY PROTOCOL:

***All samples and controls should be tested in duplicate.***

- 1) Prepare **1X GLS buffer** by diluting **4X GLS buffer** 4-fold and 0.5 M DTT 500-fold (1 mM final assay concentration) into water. For example, to prepare 10 ml, add 2.5 ml of **4X GLS buffer** and 20  $\mu$ l of 0.5 M DTT to 7.5 ml of water.
- 2) Add 20  $\mu$ l **1X GLS buffer** to each well designated "Blank."
- 3) Thaw **GLS1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Prepare **Enzyme solution (0.5 ng/ $\mu$ l GLS1)** by diluting **GLS1** in **1X GLS buffer**. Store remaining undiluted enzyme in aliquots at  $-80^{\circ}\text{C}$ . Note: **GLS1** is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
- 4) Add 20  $\mu$ l **Enzyme solution (0.5 ng/ $\mu$ l GLS1)** to each well designated "Positive Control," "Negative Control," and "Test Inhibitor."
- 5) Add 5  $\mu$ l **Inhibitor solution** to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 5  $\mu$ l of 10% DMSO in water (Inhibitor buffer). Optional: for each well designated "Negative Control," add 5  $\mu$ l **CB-839** diluted 0.1 – 0.0001  $\mu$ M in **1X GLS buffer**. Incubate at room temperature for 60 minutes (CB-839 is a potent GLS1-selective, time-dependent, slow kinetics inhibitor).
- 6) Prepare **Substrate solution** by diluting **L-Glutamine (100 mM)** 62.5-fold, **NAD<sup>+</sup> (20 mM)** 5-fold and **Coupling reagent** 600-fold in 1X GLS1 buffer. For example, to prepare 1000  $\mu$ l, add 16  $\mu$ l L-Glutamine (100 mM), 200  $\mu$ l NAD<sup>+</sup> (20 mM) and 1.66  $\mu$ l coupling reagent to 782.34  $\mu$ l 1X GLS1 buffer. Do not re-use Substrate solution.
- 7) Add 25  $\mu$ l **Substrate solution** to all wells. Read fluorescence intensity of the samples ( $\lambda_{\text{excitation}} = 340 \text{ nm}$ ;  $\lambda_{\text{emission}} = 460 \text{ nm}$ ) in an appropriate microplate reader ("t = 0 min reading"). Incubate reaction for 30 minutes at room temperature.

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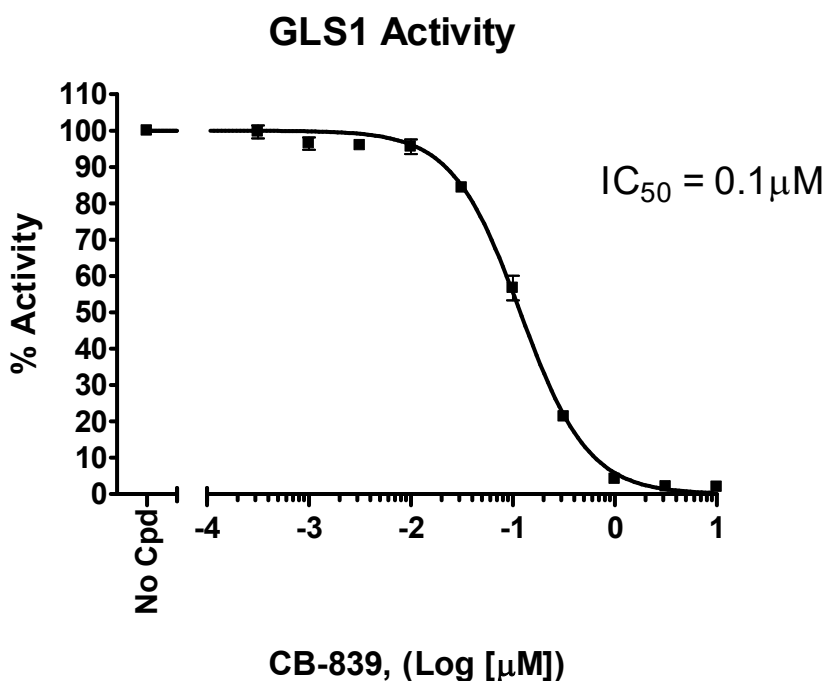
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	Positive Control	Negative Control	Test Inhibitor	Blank
Enzyme solution (0.5 ng/ $\mu$ l GLS1)	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l	-
1X GLS buffer	-	-	-	20 $\mu$ l
Inhibitor (in 1X GLS buffer)	-	-	5 $\mu$ l	-
10% DMSO in water (Inhibitor buffer)	5 $\mu$ l	-	-	5 $\mu$ l
CB-839	-	5 $\mu$ l	-	-
Substrate solution	25 $\mu$ l	25 $\mu$ l	25 $\mu$ l	25 $\mu$ l
Total	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l

- 8) Read fluorescence intensity of the samples ( $\lambda_{excitation} = 340 \text{ nm}$ ;  $\lambda_{emission} = 460 \text{ nm}$ ) ("t = 30 min reading"). Subtract background fluorescence intensity values to get net fluorescence intensity: "t = 30 min reading" - "t = 0 min reading."

#### Example of assay results:



GLS1 inhibition by CB-839, measured using the *GLS1 Inhibitor Screening Assay Kit*, BPS Bioscience Cat #79596. Fluorescence was measured using a Bio-Tek microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

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**RELATED PRODUCTS:**

<b><u>Product</u></b>	<b><u>Catalog#</u></b>	<b><u>Size</u></b>
GLS1, His-tag	71102	20 µg
GLS2, His-tag	71242	20 µg
GLS Assay Buffer	79948	10 ml
GLS2 Inhibitor Screening Assay Kit	79925	96 rxns

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