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Produktinformation

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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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	20 µg	-80 °C	Avoid multiple freeze/thaw cycles!
	L-Glutamine (100 mM)	100 µl	-20 °C	
	NAD^+ (20 mM)	1 ml	-20 °C	
	Coupling reagent	10 µl	-20 °C	
	4X GLS1 assay buffer*	2.5 ml	-20 °C	
	CB-839 (100 µM)	20 µl	-20 °C	
79685	96-well black microplate			

* 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 λexcitation = 340 nm; λemission = 460 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.

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.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Prepare **1X GLS1 buffer** by diluting **4X GLS1 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 GLS1 buffer** and 20 µl of 0.5 M DTT to 7.5 ml of water.
2) Add 20 µl **1X GLS1 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/µl GLS1)** by diluting **GLS1** in **1X GLS1 buffer**. Store remaining undiluted enzyme in aliquots at -80°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 µl **Enzyme solution (0.5 ng/µl GLS1)** to each well designated "Positive Control," "Negative Control," and "Test Inhibitor."
5) Add 5 µl **Inhibitor solution** to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 5 µl of 10% DMSO in water (Inhibitor buffer). Optional: for each well designated "Negative Control," add 5 µl **CB-839** diluted 0.1 – 0.0001 µM in **1X GLS1 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^+ (20 mM)** 5-fold and **Coupling reagent** 600-fold in 1X GLS1 buffer. For example, to prepare 1000 µl, add 16 µl L-Glutamine (100 mM), 200 µl NAD^+ (20 mM) and 1.66 µl coupling reagent to 782 µl 1X GLS1 buffer. Do not re-use Substrate solution.
7) Add 25 µl **Substrate solution** to all wells. Read fluorescence intensity of the samples (λexcitation = 340 nm; λemission = 460 nm) in an appropriate microplate reader ("t = 0 min reading"). Incubate reaction for 30 minutes at room temperature.

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

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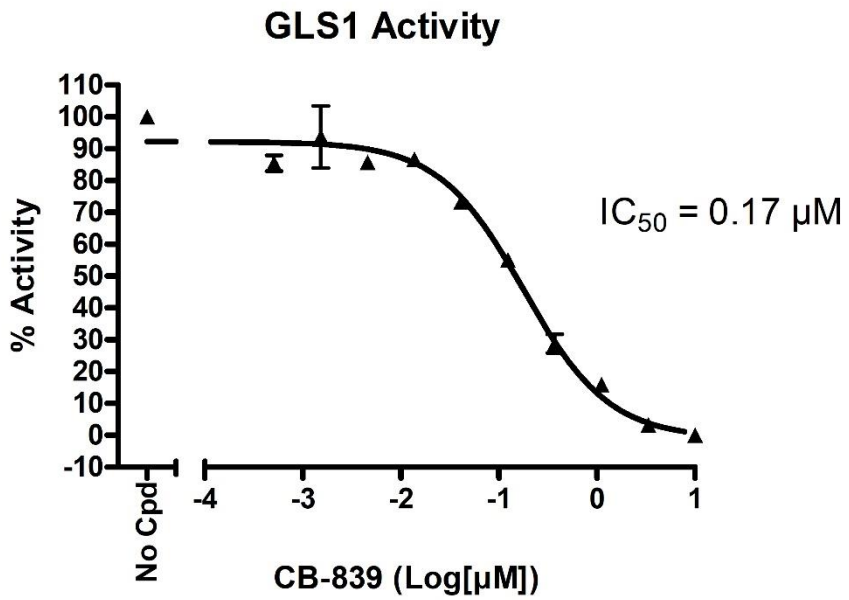
8) Read fluorescence intensity of the samples (λexcitation = 340 nm; λemission = 460 nm) ("t = 30 min reading"). Subtract background fluorescence intensity values to get net fluorescence intensity: "t = 60 min reading" - "t = 0 min reading."

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 info@bpsbioscience.com

RELATED PRODUCTS:

Product	**Catalog#**	**Size**
GLS1, His-tag	71102	20 µg
GLS2, His-tag	71242	20 µg