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Data sheet
GLS2 Inhibitor Screening Assay Kit
Catalog #79925
Size: 96 reactions

BACKGROUND: Liver-type glutaminase (GLS2) is a phosphate-activated amidohydrolase that catalyzes the hydrolysis of L-glutamine to L-glutamate and ammonia. GLS2 is related to tumor progression and cancer, including breast and hepatocellular carcinoma.

DESCRIPTION: The *GLS2 Inhibitor Screening Assay Kit* is designed to measure the hydrolase activity of GLS2 for screening and profiling applications. The GLS2 assay kit comes in a convenient 96-well format, with purified GLS2, its substrates, the Coupling reagent, and buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71242	GLS2, His-tag	30 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 GLS assay buffer	2.5 ml	-20 °C	
79685	96-well black microplate	1	Room Temp	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
0.5 M dithiothreitol (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. Xiang, Lisha, *et al.* (2013) "Knock-down of glutaminase 2 expression decreases glutathione, NADH, and sensitizes cervical cancer to ionizing radiation." *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* **1833.12:** 2996-3005.
2. Zhang, Jianbin, *et al*. (2013) "Epigenetic silencing of glutaminase 2 in human liver and colon cancers." *BMC cancer* **13.1:** 601-609.

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

1) Add 20 µl of 0.5 M DTT (not supplied) to 2.5 ml 4X GLS2 assay buffer. Prepare **1X GLS buffer** by diluting **4X GLS buffer** with DTT 4-fold into water. For example, to prepare 10 ml, add 2.5 ml of **4X GLS buffer** and 20 µl of 0.5 M DTT to 7.5 ml of water. (Final assay concentration of DTT is 1 mM).
2) Add 20 µl **1X GLS buffer** to each well designated "Blank."
3) Thaw **GLS2** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Prepare **Enzyme solution (15 ng/µl GLS2)** by diluting **GLS2** in **1X GLS buffer**. Store remaining undiluted enzyme in aliquots at -80°C. Note: **GLS2** 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 (15 ng/µl GLS2)** to each well designated "Positive Control," and "Test Inhibitor."
5) Prepare 100x concentration of test compound in DMSO. Dilute test compound 1:10 in water, and add 5 µl to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 5 µl of 10% DMSO in water (Inhibitor buffer).
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 GLS 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 GLS 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	**Test Inhibitor**	**Blank**
Enzyme solution (15 ng/µl GLS2)	20 µl	20 µl	-
1X GLS buffer	-	-	20 µl
Test Inhibitor	-	5 µl	-
10% DMSO in water (Inhibitor buffer)	5 µl	-	5 µl
Substrate solution	25 µl	25 µl	25 µl
Total	50 µl	50 µl	50 µl

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."

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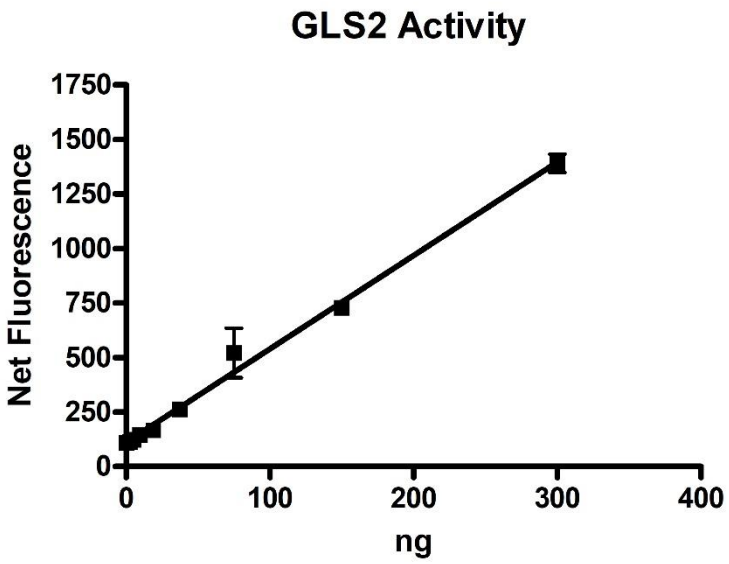
Example of assay results:



GLS2 titration measured using the *GLS2 Inhibitor Screening Assay Kit*, BPS Bioscience, #79925. 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
GLS1 Inhibitor Screening Assay Kit	79596	96 rxns.