



# SZABO SCANDIC

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- Trockeneiszuschlag
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- Expressversand

### SZABO-SCANDIC HandelsgmbH

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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 <sup>+</sup> (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  $\lambda_{excitation} = 340 \text{ nm}$ ;  $\lambda_{emission} = 460 \text{ nm}$

**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.

**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  $\mu$ l 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  $\mu$ l of 0.5 M DTT to 7.5 ml of water. (Final assay concentration of DTT is 1 mM).
- 2) Add 20  $\mu$ l **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/ $\mu$ l GLS2)** by diluting **GLS2** in **1X GLS buffer**. Store remaining undiluted enzyme in aliquots at  $-80^{\circ}\text{C}$ . 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  $\mu$ l **Enzyme solution (15 ng/ $\mu$ l 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  $\mu$ l 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).
- 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 GLS 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  $\mu$ l 1X GLS 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.

|                                       | Positive Control | Test Inhibitor | Blank      |
|---------------------------------------|------------------|----------------|------------|
| Enzyme solution (15 ng/ $\mu$ l GLS2) | 20 $\mu$ l       | 20 $\mu$ l     | -          |
| 1X GLS buffer                         | -                | -              | 20 $\mu$ l |
| Test Inhibitor                        | -                | 5 $\mu$ l      | -          |
| 10% DMSO in water (Inhibitor buffer)  | 5 $\mu$ l        | -              | 5 $\mu$ l  |
| Substrate solution                    | 25 $\mu$ l       | 25 $\mu$ l     | 25 $\mu$ l |
| Total                                 | 50 $\mu$ l       | 50 $\mu$ l     | 50 $\mu$ l |

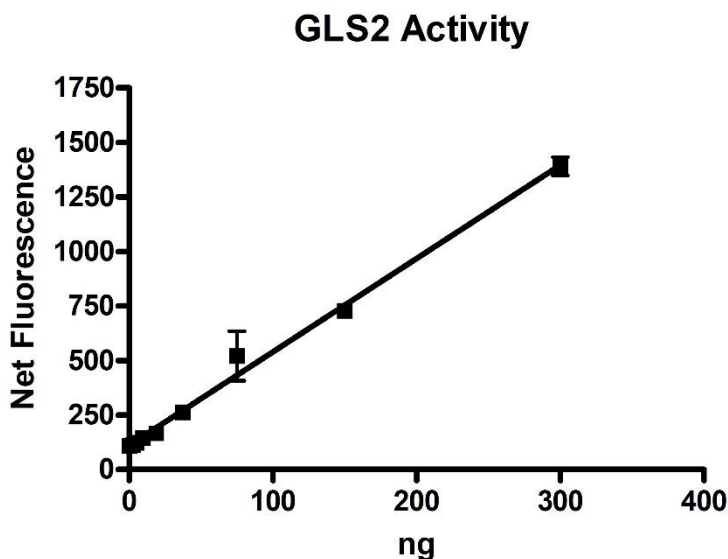
- 8) Read fluorescence intensity of the samples ( $\lambda_{\text{excitation}} = 340 \text{ nm}$ ;  $\lambda_{\text{emission}} = 460 \text{ nm}$ ) ("t = 30 min reading"). Subtract background fluorescence intensity values to get net fluorescence intensity: "t = 60 min reading" - "t = 0 min reading."

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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](mailto:info@bpsbioscience.com)

**RELATED PRODUCTS:**

| <u>Product</u>                     | <u>Catalog#</u> | <u>Size</u> |
|------------------------------------|-----------------|-------------|
| GLS1, His-tag                      | 71102           | 20 µg       |
| GLS2, His-tag                      | 71242           | 20 µg       |
| GLS1 Inhibitor Screening Assay Kit | 79596           | 96 rxns.    |

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