ab283389 – Glutaminase (GLS1) Inhibitor Screening Kit (Fluorometric)

For the screening of potential Glutaminase inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283389

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Components are stable for at least two months.

Materials Supplied

Item	Quantity	Storage Condition
Glutaminase Assay Buffer	25 mL	-20°C
GLS Dilution Buffer	200 µL	-20°C
Glutamine (GLS Substrate)	1 vial	-20°C
Converter Mix A	1 vial	-20°C
Developer Mix E	1 vial	-20°C
PicoProbe I	0.4 mL	-20°C
Active Glutaminase	20 µL	-20°C
CB-839 (GLS Inhibitor)	20 µL	-20°C

PLEASE NOTE: PicoProbe I was previously labelled as GLS1 Probe, and Glutamine (GLS Substrate) as GLS1 Substrate, and Active Glutaminase as GLS1, and Glutaminase Assay Buffer as GLS1 Assay Buffer, and Converter Mix A as Converter Mix I and GLS1 Developer, and GLS Dilution Buffer as GLS1 Dilution Buffer, and Developer Mix E as Development Enzyme Mix VI and GLS1 Enzyme Mix. The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer
- 96-well white plate with flat bottom
- Distilled water

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Glutaminase Assay Buffer: Warm to room temperature (RT) before use.

GLS Dilution Buffer: Keep on ice when in use.

Glutamine (GLS Substrate): Reconstitute in 220 µL water. Heat on a water bath at 37°C for 15 min to allow it to dissolve completely. Aliquot and store at -20°C.

PicoProbe I and Inhibitor: Thaw PicoProbe I and CB-839 (GLS Inhibitor) at RT before use. Aliquot and store at -20°C in the dark.

Converter Mix A and Developer Mix E: Reconstitute each vial with 220 µL Glutaminase Assay Buffer. Aliquot and store at -20°C.

GLS1: Always keep on ice. Aliquot into four aliquots of 5 µL each and store at -20°C. 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 Glutaminase Assay Buffer/GLS1 ASSAY BUFFER.**

Δ Note: *Keep GLS1, Converter Mix A and Developer Mix E on ice while performing the assay.*

Assay Protocol

Test Compound preparation:

1. Dissolve the test compound (S) in appropriate solvent.
2. 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 (2% V/V).
3. Add 2 µL test compound to each well of the 96-well white plate.
4. For solvent control (SC) add 2 µL of the solvent used to prepare test compound solution at its final concentration in test wells.
5. For inhibitor control (IC) add 2 µL of CB-839 (GLS Inhibitor)
6. Bring up the volume of (S), (SC) and (IC) to 50 µL with Glutaminase Assay Buffer/GLS1 Assay Buffer.
7. For the enzyme control (EC) and substrate background control (BC), add 50 µL Glutaminase Assay Buffer to a well.

Reaction Mix:

1. Dilute one aliquot of GLS1 as described above in GLS Dilution Buffer.
2. Mix enough reagents for the number of assays to be performed.
3. Add Reaction Mix to (S), (SC), (IC) and (EC).
4. 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 GLS1	2 µL	--

5. Incubate at room temperature for 10 minutes.

Substrate Mix:

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

	Substrate Mix
Glutaminase Assay Buffer	22 µL
Glutamine (GLS Substrate)	2 µL
Converter Mix A	2 µL
Developer Mix E	2 µL
PicoProbe I	2 µL

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

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

Measurement

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

Calculation:

1. Subtract "Substrate Background Control" RFU values from RFU values for all other groups to obtain background subtracted RFU values.
2. 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.
3. 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.

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

$$\% \text{ Relative activity} = \frac{slope\ of\ (test\ compound)}{slope\ of\ (enzyme\ control)} \times 100$$

Technical Support

For all technical or commercial enquiries please go to:

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