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Glutamate Dehydrogenase Inhibitor Screening Kit (Fluorometric)

rev 11/19

(Catalog # K185-100; 100 assays; Store at - 20ºC)

I. Introduction:

Glutamate dehydrogenase (GDH) (1.4.1.2) is a hexameric enzyme that catalyzes the reversible conversion of glutamate to α-ketoglutarate and ammonia while reducing NAD^+ to NADH. Glutamate can be converted to α-ketoglutarate by GDH and then fluxed into the TCA cycle where it can be used to support oxidative phosphorylation, production of lipids, or to replenish key intermediates such as oxaloacetate. It is a key enzyme in the glutamine metabolic pathway. Overexpression of GDH has been shown to promote cell proliferation, migration and invasion in several tumors such as colorectal cancer. The increased reliance on glutamine metabolism makes GDH a target for therapeutic intervention in cancer. GDH inhibitors have been shown to be toxic for cancer cells *in vitro*. In BioVision's Glutamate Dehydrogenase inhibitor screening Kit, Glutamate is deaminated by GDH producing NADH, which converts a non-fluorescent probe to a fluorescent product in the presence of enzyme mix that is detected fluorometrically at Ex/Em = 535/587 nm. The kit provides a rapid, simple, sensitive plate based test, which is also suitable for high-throughput screening of GDH inhibitors.

Glutamate —(GDH + Enzyme Mix + Probe)→ **Fluorescence (Ex/Em = 535/587 nm)**

II. Applications:

- Screening of potential inhibitors of Glutamate Dehydrogenase

III. Kit Contents:

Components	K185-100	Cap Code	Part Number
GDH Assay Buffer	25 ml	WM	K185-100-1
GDH Substrate	200 µl	Amber	K185-100-2
GDH Developer	1 vial	Red	K185-100-3
GDH Probe	400 µl	Blue	K185-100-4
GDH Enzyme	1 vial	Green	K185-100-5
GDH Inhibitor	1 vial	Orange	K185-100-6

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Distilled Water
- Glycerol

V. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials before opening. Read the entire protocol before performing the assay. Components are stable for at least two months.

- **GDH Assay Buffer:** Warm to room temperature before use.
- **GDH Substrate and Probe:** Thaw GDH substrate and Probe at room temperature before use. Aliquot and store at -20ºC in the dark.
- **GDH Developer:** Reconstitute with 220 µl GDH Buffer. Aliquot and store at -20ºC.
- **GDH Enzyme:** Reconstitute with 220 µl of 50% glycerol (50% glycerol made in water). Mix well. Aliquot and store at -20ºC. Lyophilized enzyme is stable up to 12 months at -20ºC
- **GDH Inhibitor**: Reconstitute with 44 µl water. Aliquot and store at -20ºC.

Note: Keep GDH Enzyme Mix and GDH Enzyme mix on ice while performing the assay.

VI. GDH Inhibitor Screening Assay Protocol:

1. **Test Inhibitor Preparation:** Dissolve the Test Inhibitor in appropriate solvent. Prepare at concentrations so that the volume of the Test Inhibitor solution added to a well is no more than 5 µl in the final 100 µl reaction volume per well. Add 5 µl Test Inhibitor to each well of the 96 well plate. For "Solvent Control", add 5 µl of the Solvent used to prepare Test Inhibitor solution at its final concentration in inhibitor wells. For "Inhibitor Control", add 4 µl of GDH Inhibitor and bring up the volume to 50 µl in each well by adding GDH Assay Buffer. For "Enzyme Control", add 50 µl of GDH Assay Buffer in each well.
2. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µl Mix containing:

	Reaction Mix
GDH Assay Buffer	18 µl
GDH Enzyme	2 µl

Add Reaction Mix to Test Inhibitor, Solvent Control, Inhibitor Control and Enzyme Control wells. Incubate at RT for 10 min.

3. **Substrate Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 30 µl Mix containing

	Substrate Mix
GDH Assay Buffer	24 µl
GDH Substrate	2 µl
GDH Developer	2 µl
GDH Probe	2 µl

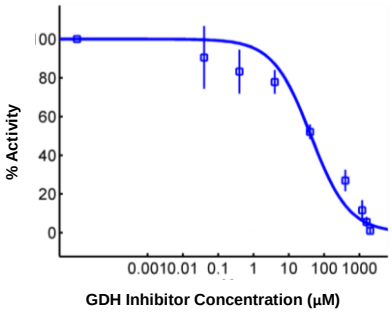
Mix well and add 30 µl of the Substrate Mix to all wells including Test Inhibitor, Solvent Control, Inhibitor Control and Enzyme Control.

4. **Measurement:** Read fluorescence in a kinetic mode at Ex/Em = 535/587 nm at 30 second intervals for 10-20 min at 37°C.
5. **Calculation:** Obtain Δ RFU for all Test Inhibitors, 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. 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.

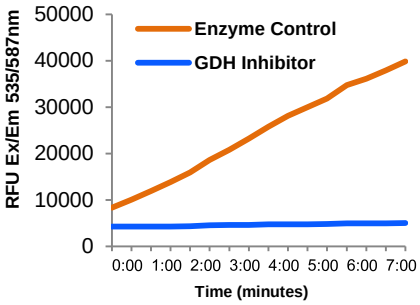
$$\% \text{ Inhibition} = \frac{[slope\ of\ (enzyme\ control) - slope\ of\ (test\ compound)]}{slope\ of\ (enzyme\ control)}\ X\ 100$$

$$\% \text{ Relative activity} = \frac{[slope\ (test\ compound)]}{slope\ (enzyme\ control)}\ X\ 100$$

a)



b)



Figures: a. Inhibition of GDH activity by GDH Inhibitor. IC_{50} of was determined to be 42 µM. **b**. Enzyme kinetics in presence and absence of GDH Inhibitor. Assays were performed using kit protocol.

VII. RELATED PRODUCTS:

Glutamate Dehydrogenase Activity Colorimetric Assay Kit (K729)
Glutamate Colorimetric Assay Kit (K629)
PicoProbe™ Glutamate Assay Kit (Fluorometric) (K413)
Alpha-Ketoglutarate Colorimetric/Fluorometric Assay Kit (K677)

FOR RESEARCH USE ONLY! Not to be used on humans.