Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Glyoxalase I Activity Assay Kit (Colorimetric)

05/17

(Catalog # K591-100; 100 assays; Store at -20°C)

I. Introduction:

Glyoxalase I (GloI, lactoylglutathione lyase, EC 4.4.1.5) is a cytosolic enzyme that participates in the Glyoxalase system. The glyoxalase system is a metabolic pathway that detoxifies α-ketoaldehydes such as methylglyoxal to D-lactic acid, via the intermediate S-D-lactoyl-glutathione (SLG). Specifically, GloI catalyzes the formation of SLG from methylglyoxal and reduced Glutathione. It is overexpressed in several human cancers such as colon, breast, prostate and melanoma. GloI also enhances resistance to antitumor agent-induced apoptosis in human leukemia cells. In addition, recent findings have linked GloI to numerous anxiety-like behaviors including psychiatric diseases (depression, schizophrenia and autism) and pain. BioVision's Glyoxalase I Activity Assay Kit utilizes the ability of an active GloI to catalyze the formation of SLG by using two GloI substrates. The formation of the SLG can be tracked using a microplate reader at OD 240 nm. Our assay kit is simple, sensitive and can detect as low as 0.05 mU of Glo I activity in biological samples.

GloI Substrate A + GloI Substrate B —(preincubation 10 min)→ Intermediate —(**Glyoxalase I**)→ SLG (OD 240 nm)

II. Applications:

- Measurement of Glyoxalase I activity in various Biological Samples/Preparations

III. Sample Type:

- Tissue Homogenates: Liver, Kidney, etc.
- Cell Lysates: U937, Hep G2 cells, etc.
- Purified Enzyme

IV. Kit Contents:

Components	K591-100	Cap Code	Part Number
GloI Assay Buffer	25 ml	WM	K591-100-1
Glyoxalase I (Lyophilized)	1 vial	Green	K591-100-2
GloI Substrate A	1.1 ml	Brown	K591-100-3
GloI Substrate B (Lyophilized)	1 vial	Yellow	K591-100-4
U.V. transparent plate (96-well)	1 plate	--	K591-100-5

V. User Supplied Reagents and Equipment:

- Microplate reader capable of absorbance measurement (OD 240 nm)
- Dounce Tissue Homogenizer (Cat. #1998 or equivalent)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **GloI Assay Buffer:** Store at either 4 ºC or -20 ºC. Bring to room temperature before use.
- **Glyoxalase I:** Reconstitute in 100 µl GloI Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **GloI Substrate A:** Aliquot and store at 4 ºC or -20 ºC. Protect from light.
- **GloI Substrate B:** Dissolve in 1.1 ml GloI Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at -20 °C. Use within two months. Freeze immediately after each use.
- **U.V. transparent plate**: Upon receiving, the plate can be stored at room temperature.

VII. Glyoxalase I Activity Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (10-50 mg) or pelleted cells (~1-2 X10^6) with 300 µl of ice-cold GloI Assay Buffer containing protease inhibitors (we suggest use PMSF) and keep on ice for 10 min. Centrifuge samples at 12,000 x *g* at 4 ºC for 10 min. collect the supernatant and keep on ice.
2. **Substrate Mix Preparation:** Mix enough reagents for the number of assays to be performed in a 1.5 ml centrifuge tube. For each well, prepare a total of 50 µl Substrate Mix containing the following components. Mix well.

	Substrate Mix*
GloI Assay Buffer	30 µl
GloI Substrate A	10 µl
GloI Substrate B	10 µl

Mix. Well. Incubate the Substrate Mix at room temperature for 10 min, **avoid light**. After the 10 minpre-incubation, add 50 µl of the Substrate Mix to the wells of the provided **U.V. transparent plate** and labeled as Samples, Reagent Background Control, and Positive Control respectively.

***Note:**

1) The 10 min-preincubation time is *mandatory*. It allows the formation of a product (intermediate form) via non enzymatic reaction, which serves as GloI substrate.
2) The Substrate Mix should be freshly prepared, kept on ice and used within 2 hours, since it will slowly isomerize to SLG non-enzymatically. *Do no store unused Substrate Mix. Prepare enough reagents for the number of experiments to be performed.*

3. **Reaction Development:** For Sample well(s): add 2-10 µl prepared samples (Step 1); For Reagent Background Control well: add same volume of GloI Assay Buffer; For Positive Control well: add 2-10 µl Reconstituted Glyoxalase I. Bring the total volume to 100 µl of each well, mix thoroughly.

	Sample/Positive Control	Background Control
Sample/Positive Control	2-10 µl	---
GloI Assay Buffer	up to 100 µl	100 µl

Note: We suggest using 3-5 different amounts of the samples to ensure the kinetic responses are within the linear range.

4. **Measurement:** Immediately measure absorbance (OD 240 nm) in kinetic mode at room temperature for 10-20 min.
5. **Calculation:** Take the absorbance (OD 240 nm) at two time points (t_1 and t_2) in the linear range. To determine Activity, use the following equation:

$$\textbf{Sample GloI Activity} = \frac{\left(\frac{\Delta A_{240nm}}{\Delta t}Test - \frac{\Delta A_{240nm}}{\Delta t}Reagent\ Background\right) x\ (0.1)\text{xD}}{3.37\ x\ 0.29\ x\ \text{V x P}} = \textbf{Units/mg protein}$$

Where: **0.1** = Reaction volume (ml)
3.37 = millimolar extinction coefficient of SLG ($mM^{-1}cm^{-1}$)
0.29 = light path (cm)
V = Sample volume added into the reaction well (ml)
P = Initial Sample Concentration in mg-protein/ml (mgP/ml)
D = Sample Dilution Factor (D = 1 for undiluted samples)

Unit Definition: One unit of Glyoxalase I activity is the amount of enzyme that converts 1µmol of SLG per min under the assay conditions at 25 ºC

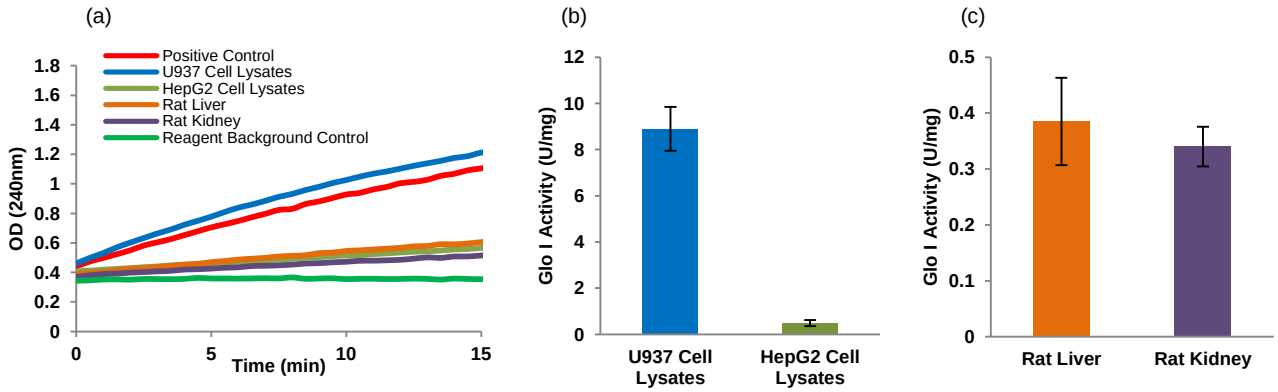

Figure: (a) Measurement of GloI activity in U937 Cell Lysate (0.6 µg Protein), Hep G2 Cell Lysates (2 µg Protein), Rat Liver tissue extracts (1.5 µg Protein), Rat Kidney tissue extracts (2 µg Protein) & Positive Control. (b-c) Referenced GloI Activity in U937 Cell Lysates, HepG2 Cell Lysates, Rat Liver tissue extracts and Rat Kidney tissue extracts. All assays were performed following kit protocol.

VIII. RELATED PRODUCTS:

Human Recombinant Glyoxalase I (6389)
Glyoxalase II, Human Recombinant (P1072)
Glyoxalase II Activity Kit (K460)
EZDetect™ Aldo-keto Reductase Activity Assay Kit (Colorimetric) (K847)
Aldehyde Dehydrogenase Activity Colorimetric Assay Kit (K731)
Dounce Tissue Homogenizer (1998)

FOR RESEARCH USE ONLY! Not to be used on humans