Glyoxalase II Activity Kit (Colorimetric) (BN00698)

(Catalog # BN00698 100 assays; Store at -20°C)

I. Introduction:

Glyoxalase II (GloII, hydroxyacylglutathione hydrolase, EC 3.1.2.6) is one of the two enzymes in the Glyoxalase system that is ubiquitously expressed in mammalian, plants and bacteria. The Glyoxalase system is a biological pathway that detoxifies cells by metabolizing α-ketoaldehydes such as methylglyoxal, a cytotoxic byproduct of lipids and glucose metabolism. GloII fulfills the terminal step in the glyoxalase system: It catalyzes the hydrolysis of *S*-D-lactoyl-glutathione (SLG) to reduced glutathione and D-lactic acid. The alteration of GloII activity in cell growth cycle, cell differentiation, phagocyte activation and *diabetes mellitus* development of implies this enzyme plays a wide-ranging and important role in cell function and disease progression. Research areas of current interest in Glo-II include diabetes, and cancer therapy. Assay Genie's Glyoxalase II Activity Kit utilizes the ability of an active Glo-II to cleave a substrate while producing D-lactate. The produced D-Lactate reacts with the chromophore generating a stable signal that can be easily quantified at 450 nm using a microplate reader. Our assay kit is simple, sensitive and can detect as low as 0.6 mU/ml of Glo-II activity in biological samples.

GloII Substrate —(Glyoxalase II)→ D-lactate

D-lactate + GloII Probe —(Enzyme Mix)→ Absorbance (OD 450nm)

II. Applications:

- Measurement of Glyoxalase II activity in various biological samples/preparations

III. Sample Type:

- Tissue homogenates and cell lysates: Liver, HepG2 cells, etc.
- Whole Blood, Red Blood cells
- Purified Enzyme or protein preparations

IV. Kit Contents:

Components	BN00698	Cap Code	Part Number
GloII Assay Buffer	25 ml	WM	BN00698-1
Enzyme Mix (Lyophilized)	1 vial	Green	BN00698-2
GloII Substrate (Lyophilized)	1 vial	Brown	BN00698-3
GloII Probe (Lyophilized)	2 vials	Red	BN00698-4
GloII Positive Control	8 µl	Blue	BN00698-5
D-Lactate Standard (100 mM)	100 µl	Yellow	BN00698-6

V. User Supplied Reagents and Equipment:

- Microplate reader
- 96-well clear plate
- Ammonium Sulfate Solution (Saturated, 4.1 M)
- Dounce Tissue Homogenizer

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.

- **GloII Assay Buffer:** Store at either 4 ºC or -20 ºC. Bring to room temperature before use.
- **Glyoxalase II and D-Lactate Standard:** Store at -20°C. Keep on ice while in use. Use within two months.
- **Enzyme Mix:** Dissolve in 220 µl GloII Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20 °C. Use within two months. Use within two months.
- **GloII Substrate:** Reconstitute with 1.2 ml **dH_2O** and mix thoroughly. Aliquot and store at -20 °C. Use within two months.
- **GloII Probe:** Reconstitute 1 vial of GloII Probe with 220 µl GloII Assay Buffer and mix thoroughly. Dissolve vial contents when needed. Store at 20 °C and use within two months.

VII. Glyoxalase II Activity Assay Protocol:

1. Sample Preparation:

Tissue and Cell Samples: Rapidly homogenize tissue (10-20 mg) or pelleted cells (~1-2 x 10^6) with 300 µl ice-cold GloII Assay Buffer containing protease inhibitors and keep on ice for 10 min. Centrifuge samples at 12,000 x g at 4 ºC for 10 min. and collect the supernatant. Remove endogenous interference from tissue and cell samples by using the ammonium sulfate precipitation method: Aliquot samples (2-100 µl) to clean centrifuge tubes, add saturated ammonium sulfate (about 4.1 M at room temperature) to a final concentration 3.2 M, set on ice for 20 min., mix well, and spin down at 14,000 rpm for 5 min. (*Do not vortex*), discard the supernatant. Repeat the same procedure for one more time and suspend the pellet to the original volume of Assay Buffer.

Red Blood Cells and Whole Blood: Dilute Red Blood Cells (1:100 fold) or Whole Blood (1:50 fold) with ice-cold GloII Assay Buffer to lyse cells. Centrifuge at 6,000 x g at 4 ºC for 10 min. and collect the supernatant.

Add 2-10 µl of sample into desired well(s) to a 96-well clear plate labeled as Sample and add the same volume of GloII buffer to well(s) labeled as Reagent Background Control. For Positive Control, prepare a 1:200 dilution of GloII Positive Control using GloII Assay Buffer. Add 4-10 µl of Diluted GloII into desired well(s). Adjust the volume of Sample, Reagent Background Control and Positive Control wells to **50 µl/well** with GloII Assay Buffer.

Note:

a. Endogenous substances from **cell or tissue extracts** might interfere with the assay generating background. Prepare Sample Background Control well to correct these interferences. Same amount of sample can be tested in the absence of Enzyme Mix (see Step 4) as **Sample Background Control**. Then, the background reading can be subtracted from the D-lactate reading.

b. We suggest using 3-5 different amounts of the samples per well to ensure the readings are within the standard curve range and the changes of velocity are within the lineal range.

2. **Standard Curve Preparation:** Dilute the 100 mM D-Lactate Standard to 1 mM by adding 10 µl of the Standard to 990 µl of GloII Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl of Diluted D-Lactate Standard into a series of wells. Adjust volume to **50 µl/well** with GloII Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the D-lactate Standard.

3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing the following components. Mix well before use:

	Reaction Mix	*Sample Background Control Mix
GloII Assay Buffer	34 µl	36 µl
GloII Substrate	10 µl	10 µl
GloII Probe	4 µl	4 µl
Enzyme Mix	2 µl	---

Add 50 µl of the Reaction Mix to each well containing the D-Lactate Standard, Sample(s), Reagent Background Control and GloII Positive Control.

* For Background Correction of cell or tissue extracts, add 50 µl of Sample Background Control Mix (without Enzyme Mix) to Sample Background Control well and mix well (see Step 1, Note).

4. **Measurement:** Measure absorbance (OD 450 nm) in kinetic mode at room temperature for 40 min.

5. **Calculation:** Subtract 0 Standard Reading from all Standard Readings. Plot a Standard Curve of OD 450 nm vs. nmol/well D-lactate and obtain the slope of the curve; apply Sample ΔOD and Reagent Background Control ΔOD or Sample Background Control ΔOD to D-lactate Standard Curve to obtain the corresponding amounts of D-lactate formed. Calculate the Background-Corrected Samples by subtracting sample background control from sample well. (**Note:** If sample background is significant, apply this to correct its corresponding sample). Calculate the activity of GloII in the sample as:

$$\text{Sample GloII Activity} = B/(\Delta t \times V) \times D = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B** = D-lactate from Standard Curve (nmol)
Δt = Reaction time (min.)
V = Sample volume added into the reaction well (ml)
D = Sample Dilution Factor (D=1 when samples are undiluted)

GloII specific activity can be expressed as U/mg of protein.

Unit Definition: One unit of GloII activity is the amount of enzyme that catalyzes the release of 1 µmol of D-Lactate per min from the substrate under the assay conditions at room temperature.

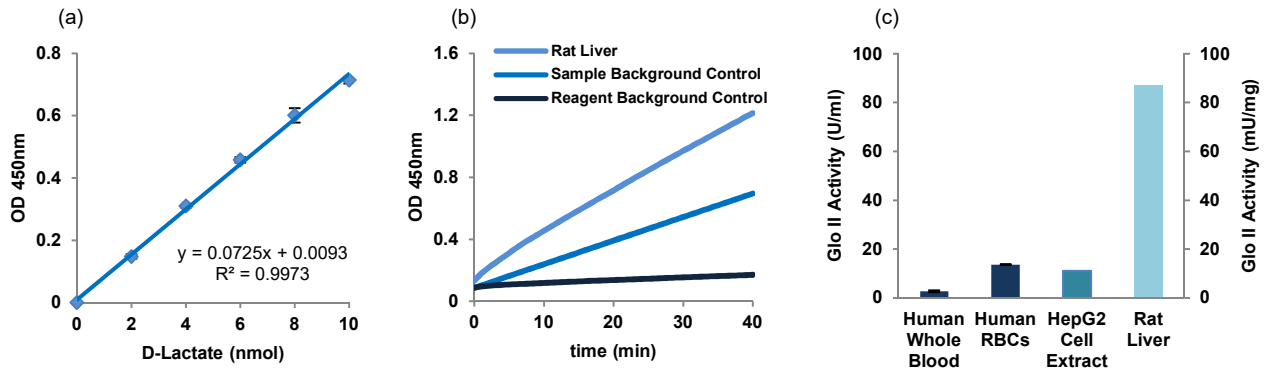

Figure: (a) D-Lactate Standard Curve, results from multiple experiments. (b) GloII Activity in Rat Liver tissue extracts (1.5 µg protein). (c) Measurement of GloII activity in Human Whole Blood (6 µl, 1: 50 dilution), Human Red Blood Cells (4 µl, 1:100 dilution), HepG2 Cell Lysates (2 µg protein) and Rat Liver tissue extracts (1.5 µg protein). All assays were performed following kit protocol.

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