# ELISA PRODUCT INFORMATION & MANUAL

# Glyoxalase 1 Activity Activity Assay Kit (Colorimetric)

## *NBP2-59746*

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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## Table of Contents

# 1. Overview

Glyoxalase 1 Activity Activity Assay Kit (Colorimetric) (NBP2-59746) utilizes the ability of an active Glyoxalase I (GloI) to catalyze the formation of S-D- lactoylglutathione (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 GloI activity in biological samples.

## 2. Protocol Summary

Prepare all samples and controls as instructed.

Prepare the Substrate Mix as instructed using GloI assay Buffer and GloI Substrate A and B.

Mix Well and incubate Substrate Mix at room temperature for 10 minutes in the dark.

Add 50 µL of the substrate Mix to the wells of containing samples and controls.

Immediately measure the absorbance (OD 240 nm) in kinetic mode at room temperature for 10-20 minutes.

Calculate the activity using the given formula in the Data analysis section.

## 3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.

- For typical data produced using the assay, please see the assay kit datasheet on our website.

## 4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

| Item | Quantity | Storage condition |
|---|---|---|
| Glol Assay Buffer | 25 mL | -20°C |
| Glyoxalase I (Lyophilized) | 1 vial | -20°C |
| Glol Substrate A | 1.1 ml | -20°C |
| Glol Substrate B (Lyophilized) | 1 vial | -20°C |
| U.V. transparent plate (96-well ) | 1 plate | -20°C |

## 5. Materials Required, Not Supplied

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

- Microplate reader capable of absorbance measurement (OD 240 nm).
- Dounce Tissue Homogenizer.

## 6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

### 6.1 GloI Assay Buffer:

Ready to use as supplied. Bring to room temperature (RT) before use. Store at either 4ºC or -20ºC.

### 6.2 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.

### 6.3 GloI Substrate A:

Aliquot and store at 4 °C or -20 °C. Protect from light.

### 6.4 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.

### 6.5 U.V. transparent plate:

Upon receiving, the plate can be stored at room temperature.

## 7. Sample Preparation

- Rapidly homogenize tissue (10-50 mg) or pelleted cells (~1-2 x$10^6$) 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 °C for 10 min collect the supernatant and keep on ice.

## 8. Assay Procedure

**8.1** Mix enough reagents for the number of assays to be performed in a 1.5 mL centrifuge tube**.**

**8.2** 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 |

**8.3** Mix well. Incubate the Substrate Mix at room temperature for 10 min, avoid light.

**8.4** After the 10 min pre-incubation, add 50 µL of the Substrate Mix to the wells of the provided U.V. transparent plate labelled as Samples, Reagent Background Control, and Positive Control respectively.

∆**Note:** 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.

∆**Note:** The Substrate Mix should be freshly prepared, kept on ice and used within 2 hours, since it will slowly isomerize to SLG nonenzymatically. Do no store unused Substrate Mix. Prepare enough reagents for the number of experiments to be performed.

**8.5** **Reaction Development:** For Sample well(s): add 2-10 µL prepared samples. For Reagent Background Control well add same volume of GloI Assay Buffer. For Positive Control well: add 2-10 µL Reconstituted Glyoxalase I.

**8.6** Bring the total volume of each well to 100 µL using GloI Assay Buffer.

| | **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.

**8.7** **Measurement:** Immediately measure absorbance (OD 240 nm) in kinetic mode at room temperature for 10-20 min.

## 9. Data Analysis

**9.1** Take the absorbance (OD 240 nm) at two time points (t1 and t2) in the linear range. To determine Activity, use the following equation:

$$Activity = \frac{\left(\frac{\Delta A_{240nm}}{\Delta t} Test - \frac{\Delta A_{240nm}}{\Delta t} Reagent\ Background\right) \times (0.1) \times D}{3.37 \times 0.29 \times V \times P} = \frac{Units}{m} protein$$

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 °C.

## 10. Typical Data

Typical data provided **for demonstration purposes only**.

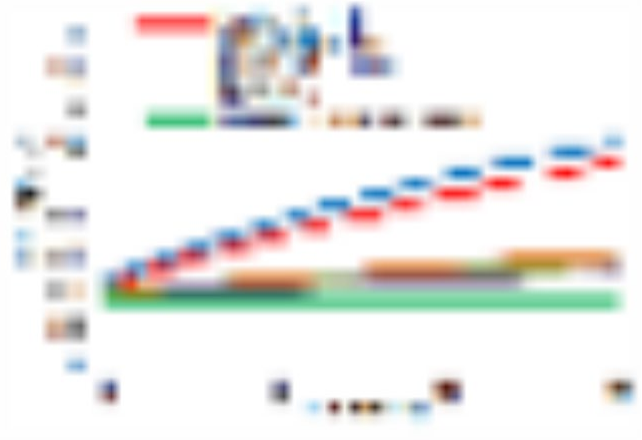

**Figure 1**. Measurement of GloI activity in U937 Cell Lysate (0.6 µg Protein), Hep G2 Cell Lysates (2 µg Protein), Rat Liver tissue extracts (1.5 µg Protein), Rat Kidney tissue extracts (2 µg Protein) & Positive Control.

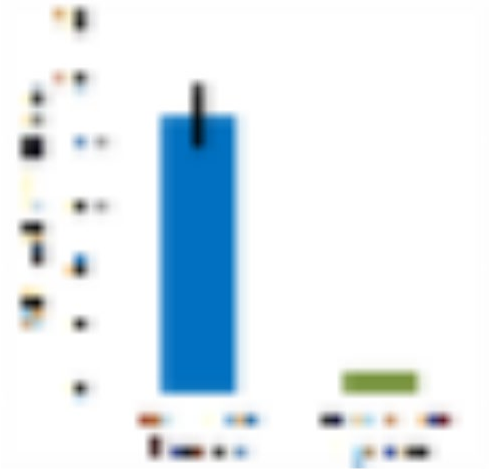

**Figure 2:** Referenced GloI Activity in U937 Cell Lysates, HepG2 Cell Lysates, Rat Liver tissue extracts and Rat Kidney tissue extracts. All assays were performed following kit protocol.

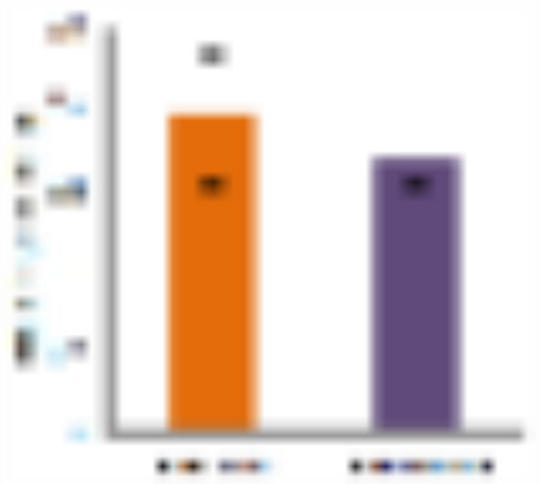

**Figure 3.** Referenced GloI Activity in U937 Cell Lysates, HepG2 Cell Lysates, Rat Liver tissue extracts and Rat Kidney tissue extracts. All assays were performed following kit protocol.

## 11. Notes