

Glyoxalase I (GLO-1) Assay Kit (Colorimetric)

LS-K216-100 (100 Tests) • See Storage Conditions Below



Introduction

GLYOXALASE I (GLO-1), a lactoylglutathione lyase also known as methylglyoxalase, aldoketomutase, ketone-aldehyde mutase, and (R)-S-lactoylglutathione methylglyoxal-lyase, is an enzyme that catalyzes the isomerization of hemithioacetal adducts which are formed in spontaneous reactions between glutathionyl groups and aldehydes. The primary physiological function of glyoxalase I is the detoxification of methylglyoxal, a reactive 2-oxoaldehyde that is cytostatic at low concentrations and cytotoxic at millimolar concentrations. Glyoxalase I is a target for the development of pharmaceuticals against bacteria, protozoans and human cancer. Simple, direct and automation-ready procedures for measuring GLO-1 activity in biological samples are highly desirable in research and drug discovery. This Glyoxalase I assay kit provides a sensitive and convenient Method for GLO-1 activity determination. The Method involves monitoring the increase in the product of the GLO-1 reaction, S-lactoylglutathione, by measuring the change in absorbance at 240 nm.

Key Features

- Sensitive and accurate. Detection limit 4 U/L GLO-1 activity.
- Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- Direct Assays: GLO-1 activity in enzyme preparations or biological samples.
- Drug Discovery/Pharmacology: effects of drugs on GLO-1 activity.

Components

Component	K216-100
	100 Tests
Assay Buffer (pH 6.6)	20 mL
Substrate	1 mL
96 well UV Titer Plate	1 Plate
Cosubstrate	1 mL

Materials Not Supplied

Perchloric Acid (e.g. Sigma Cat. No. 311413), pipetting devices and accessories and plate reader.

Storage

Kit is shipped at room temperature. Store the plate at room temperature and other components at -20°C. Shelf life: 12 months after receipt.

FOR RESEARCH USE ONLY! Not for use in humans.

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Assay Procedure

Procedure for Pure Enzyme Preparations

1. Bring all reagents to room temperature prior to assay.
2. Add 40 μ L of each sample to separate wells of the 96 well UV titer plate. Also, include one blank (sample buffer without GLO-1) well per assay run.
3. Prepare Working Reagent for all wells by mixing per well: 160 μ L Assay Buffer with 8 μ L Substrate and 8 μ L Cosubstrate. Add 160 μ L Working Reagent to each well.
4. Read the optical density at 240 nm at $t=0$ min and again at $t=10$ min. If measuring low GLO-1 activities, longer reaction times can be used.

Procedure for Proteinous Samples

1. Each sample requires 2 tubes: one for the GLO-1 Reaction and one for the Sample Blank. Add 40 μ L of each sample (serum samples should be diluted at least 2 $\times$ with assay buffer) to 2 separate Eppendorf tubes.
2. Prepare Working Reagent for all tubes by mixing per tube: 160 μ L Assay Buffer with 8 μ L Substrate and 8 μ L Cosubstrate.
3. GLO-1 Reaction. Add 160 μ L Working Reagent to the GLO-1 Reaction tubes. Incubate for 20 min at room temperature.
4. Protein Precipitation of GLO-1 Reaction. After 20 min incubation, add 70 μ L 4 M Perchloric Acid to each GLO-1 reaction tube, vortex to mix and chill for 15 min on ice. (Note: It is important that the protein precipitation is carried out on ice.) After 15 min centrifuge samples for 5 min at 14,000 rpm and transfer 200 μ L of each clear supernatant to separate wells of the 96 well UV titer plate.
5. Sample Blank. Add 70 μ L 4 M Perchloric Acid to each Sample Blank tube, vortex to mix and chill for 15 min on ice. After 15 min, add 160 μ L Working Reagent to each Sample Blank tube. Vortex to mix and chill on ice for 15 min. (Note: It is important that the sample is deproteinated prior to adding the Working Reagent.) After the second chill, centrifuge the sample blanks for 5 min at 14,000 rpm and transfer 200 μ L of each clear supernatant to separate wells of the 96 well UV titer plate.
6. Read optical density at 240 nm.

Calculations

For pure samples glyoxalase activity is calculated as follows:

$$\text{GLO-1} = \frac{\text{OD}_{10} - \text{OD}_0}{\epsilon \times l} \times \frac{V_T}{t} \times \frac{1}{V_S} = 350 \times (\text{OD}_{10} - \text{OD}_0) \text{ (U/L)}$$

where OD_{10} and OD_0 are the optical density values of the sample taken at 10 min and 0 min respectively. V_T is the total reaction volume (0.2 mL), V_S is the sample volume (40 μ L), ϵ is the S-lactoylglutathione extinction coefficient (3.37 mM⁻¹cm⁻¹), l is the path length (0.425 cm for 0.2 mL in provided plate) and t is the reaction time (10 min).

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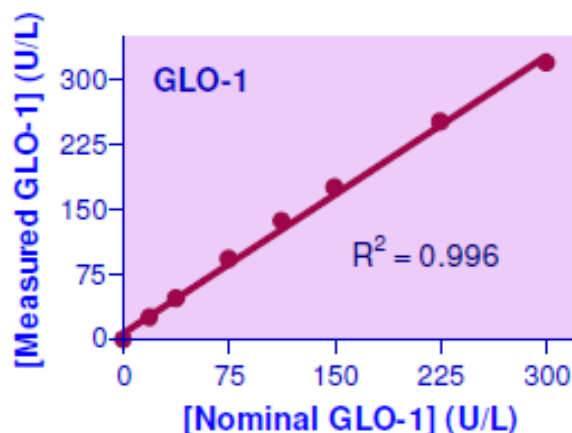
For proteinous samples glyoxalase activity is calculated as follows:

$$\text{GLO-1} = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\epsilon \times l} \times \frac{V_T}{t} \times \frac{1}{V_s} \times 1.35 \times n$$
$$= 175 \times (\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}) \times 1.35 \times n \text{ (U/L)}$$

where $\text{OD}_{\text{SAMPLE}}$ and OD_{BLANK} are the optical density values of the sample and sample blank respectively. t is the reaction time (20 min), 1.35 is the dilution factor for the deproteinization step and n is the dilution factor if a sample dilution is required.

Unit definition: 1 unit of Glyoxalase-1 forms 1 μmole of S-lactoylglutathione from methylglyoxal and reduced glutathione per minute at pH 6.6 and 25°C.

Sample Data



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