Technical Manual

ChromaDazzle Glyoxalase I Activity Assay Kit

Catalogue Code: BA0026

Pack Size: 100 assays

Research Use Only

DESCRIPTION

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. The Assay Genie ChromaDazzle Glyoxalase I Activity 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.

Simple and high-throughput. The procedure involves incubation of the provided substrate with the sample in a microplate. Can be readily automated as a high-throughput assay for 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.

KIT CONTENTS

Assay Buffer (pH 6.6):	20 mL	**Substrate:**	1 mL
96 well UV Titer Plate	1 Plate	**Cosubstrate:**	1 mL

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

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURE FOR PURE ENZYME PREPARATIONS

1. Bring all reagents to room temperature prior to assay.
2. Add 40 µL 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 µL Assay Buffer with 8 µL Substrate and 8 µL Cosubstrate. Add 160 µL 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 µL of each sample (serum samples should be diluted at least 2× with assay buffer) to 2 separate Eppendorf tubes.
2. Prepare Working Reagent for all tubes by mixing per tube: 160 µL Assay Buffer with 8 µL Substrate and 8 µL Cosubstrate.
3. *GLO-1 Reaction*. Add 160 µL 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 µL 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 µL of each clear supernatant to separate wells of the 96 well UV titer plate.

5. *Sample Blank*. Add 70 µL 4 M Perchloric Acid to each Sample Blank tube, vortex to mix and *chill* for 15 min on ice. After 15 min, add 160 µL 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 µL of each clear supernatant to separate wells of the 96 well UV titer plate.
6. Read optical density at 240 nm.

CALCULATION

For pure samples glyoxalase activity is calculated as follows:

$$\text{GLO-1} = \frac{OD_{10} - OD_0}{\varepsilon \times l} \times \frac{V_T}{t} \times \frac{1}{V_S} = 350 \times (OD_{10} - OD_0)\ (\text{U/L})$$

where **OD10** and $\mathbf{OD_0}$ are the optical density values of the sample taken at 10 min and 0 min respectively. $\mathbf{V_T}$ is the total reaction volume (0.2 mL), $\mathbf{V_S}$ is the sample volume (40 µL), **e** is the S-lactoylglutathione extinction coefficient (3.37 $mM^{-1}cm^{-1}$), ***l*** is the path length (0.425 cm for 0.2 mL in provided plate) and ***t*** is the reaction time (10 min).

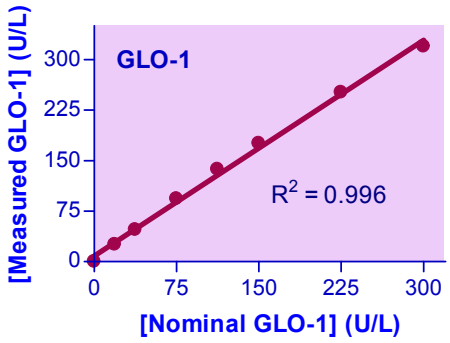
For proteinous samples glyoxalase activity is calculated as follows:

$$\text{GLO-1} = \frac{OD_{SAMPLE} - OD_{BLANK}}{\varepsilon \times l} \times \frac{V_T}{t} \times \frac{1}{V_S} \times 1.35 \times n$$

$$= 175 \times (OD_{SAMPLE} - OD_{BLANK}) \times 1.35 \times n \quad (\text{U/L})$$

where **ODSAMPLE** and $\mathbf{OD_{BLANK}}$ 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 deproteination 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.



MATERIALS REQUIRED, BUT NOT PROVIDED

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

REFERENCES

[1]. Davis, KA and Williams, GR. (1969) Glyoxalase I, a lyase or an oxidoreductive isomerase? Can. J. Biochem 47: 553-6.
[2]. Ditzen, C et al. (2006) Protein biomarkers in a mouse model of extremes in trait anxiety. Mol Cell Proteomics 5: 1914-20.
[3]. Strzinek, RA. et al. (1972) The purification and characterization of liver glyoxalase I from normal mice and from mice bearing a lymphosarcoma. Cancer Res 32:2359-64.

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