3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Glyoxalase I Activity Assay Kit

Catalog Number **MAK114**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Glyoxalase I (GLO-1) is a lactoylglutathione lyase also known as methylglyoxalase, aldoketomutase, ketone-aldehyde mutase, and (R)–S-lactoylglutathione methylglyoxal–lyase. It 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 cancer.

The Glyoxalase I Activity Assay kit provides a simple and direct procedure for measuring GLO-1 activity in a variety of samples such as enzyme preparations or biological samples. In this assay, the GLO-1-mediated production of S-lactoylglutathione is measured by monitoring the change in absorbance at 240 nm. One unit of Glyoxalase I is the amount of enzyme that will convert 1.0 μmole of S-lactoylglutathione from methylglyoxal and reduced glutathione per minute at pH 6.6 and 25 °C.

Components

The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer Catalog Number MAK114A	20 mL
96 well UV Titer Plate Catalog Number MAK114B	1 EA
Substrate Catalog Number MAK114C	1 mL
Cosubstrate Catalog Number MAK114D	1 mL

Reagents and Equipment Required but Not Provided.

- Ultraviolet Spectrophotometric multiwell plate reader
- Perchloric Acid (Catalog Number 311413 or equivalent, optional for biological proteinaceous samples)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped at room temperature. The plate can be stored at room temperature. All other components should be stored at –20 °C.

Preparation Instructions

Briefly centrifuge vials before opening. Bring all reagents to room temperature prior to assay.

Procedure

For Enzyme Samples

1. Transfer 40 μL of each sample to separate wells of the 96 well UV multiwell plate. Transfer 40 μL of Assay Buffer into a separate well to serve as an assay blank.

2. Prepare the Master Reaction Mix according to the scheme in Table 1. 160 μL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Assay Buffer	160 μL
Substrate	8 μL
Cosubstrate	8 μL

3. Add 160 μL of the Master Reaction Mix to each of the wells.

4. Read the initial absorbance at 240 nm [$(A_{240})_{initial}$]. Incubate the reaction at room temperature for an additional 10 minutes. Measure the final absorbance at 240 nm [$(A_{240})_{final}$]. If enzyme activity values are low, longer reaction times can be used.

For Biological Proteinaceous Samples

1. Transfer 40 µL of each sample into two separate microcentrifuge tubes. One tube will be the sample reaction and one tube will be the sample blank. Serum samples should be diluted at least 2-fold with assay buffer.

2. Prepare the Master Reaction Mix according to the scheme in Table 2. 160 µL of the Master Reaction Mix is required for each reaction.

Table 2.
Master Reaction Mix

Reagent	Volume
Assay Buffer	160 µL
Substrate	8 µL
Cosubstrate	8 µL

3. Add 160 µL of the Master Reaction Mix to each of the sample tubes. Do not add to the sample blank tubes. Incubate the samples for 20 minutes at room temperature.

4. After 20 minute incubation, precipitate protein by adding 70 µL of 4 M perchloric acid to each of the sample tubes. Do not add perchloric acid to the sample blank tubes. Vortex to mix and incubate for 15 minutes on ice. It is critical that the protein precipitation step be carried out on ice.

5. Following incubation, centrifuge the samples for 5 minutes at 14,000 rpm. Transfer 200 µL of cleared supernatant to separate wells of the 96 well UV multiwell plate.

6. Add 70 µL of 4 M perchloric acid to each of the sample blank tubes. Vortex to mix and incubate for 15 minutes on ice.

7. After 15 minutes, add 160 µL of the Master Reaction Mix to each of the sample blank tubes. Vortex to mix and incubate for 15 minutes on ice. It is critical that the sample blanks be deproteinated prior to adding the Master Reaction Mix.

8. Centrifuge the sample blanks for 5 minutes at 14,000 rpm. Transfer 200 µL of cleared supernatant to separate wells of the 96 well UV multiwell plate.

9. Read the absorbance at 240 nm (A_{240}).

Calculations

The Glyoxalase I activity of a sample may be determined by the following equations:

Enzyme Samples:

$$\text{Activity} = \frac{(A_{240})_{final} - (A_{240})_{initial}}{\varepsilon \times \iota} \times \frac{V_T}{t} \times \frac{1}{V_s} =$$

$$= 350 \times [(A_{240})_{final} - (A_{240})_{initial}] \text{ units/L}$$

ε = extinction coefficient for S-lactoylglutathione ($3.37\ mM^{-1}cm^{-1}$)
ι = pathlength (0.425 cm for 0.2 mL in provided plate)
V_T = total reaction volume (0.2 mL)
t= reaction time (10 minutes)
V_S= sample volume (40 µL)

Biological Proteinaceous Samples:

$$\text{Activity} = \frac{(A_{240})_{sample} - (A_{240})_{blank}}{\varepsilon \times \iota} \times \frac{V_T}{t} \times \frac{1}{V_s} \times 1.35 \times n =$$

$$= 175 \times [(A_{240})_{sample} - (A_{240})_{blank}] \times 1.35 \times n \text{ units/L}$$

ε = extinction coefficient for S-lactoylglutathione ($3.37\ mM^{-1}cm^{-1}$)
ι = pathlength (0.425 cm for 0.2 mL in provided plate)
V_T = total reaction volume (0.2 mL)
t= reaction time (10 minutes)
V_S= sample volume (40 µL)
1.35 = dilution factor for the deprotienation step
n = dilution factor if required

One unit of Glyoxalase I is the amount of enzyme that will convert 1.0 µmole of S-lactoylglutathione from methylglyoxal and reduced glutathione per minute at pH 6.6 and 25 °C.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	Use plate included in kit or plate compatible with UV assays
Samples with erratic readings	Samples prepared in incompatible buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further

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