Glycolate Oxidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1103

(Version 1.5C)

Detection and Quantification ofGlycolate Oxidase(GOX) Activityin Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Glycolate oxidase is a member of the superfamily of the a-hydroxy acid oxidases (HAO), enzymes that are present in both plants and animals. It catalyzes the FMN-mediated oxidation of glycolate to glyoxylate and glyoxylate to oxalate with reduction of oxygen to hydrogen peroxide.

Glycolate Oxidase ActivityColorimetric Microplate Assay Kitprovides a simple and direct procedure for measuring glycolate oxidase activity in a variety of samples.The assay is initiated with the enzymatic oxidization of glycolic acid byglycolate oxidase. The enzyme catalysed reaction product glyoxylic acid reacts with phenylhydrazine,glyoxylate phenylhydrazonecan be measured at a colorimetric readout at 500 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Dye Reagent I	Powderx 1	4 °C, keep in dark
Dye Reagent II	Powderx 1	4 °C, keep in dark
Dye Reagent I Diluent	10 mlx 1	4 °C
Substrate	Powderx 1	4 °C
Standard	Powderx 1	4 °C
Stop Solution	5 mlx 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 500 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 50 mmol/L of standardstock solution.Keep in dark for immediate use or store at -20°C for 1 week after reconstitution.Then dilute to 5mmol/Lstandard top solutionby adding 0.1 ml stock solution into 0.9 ml distilled water**.** Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078mmol/L.

Substrate: Briefly centrifuge prior to opening. Add 2 ml distilled water to dissolve before use. Store at 4 °C for 1-2 months or -20°C for 1 year after reconstitution.

Dye Reagent I: Add 10 mlDye Reagent I Diluent to dissolve before use.Keep in darkfor immediate use within 2 hours. If the color change to yellow, it may be out of work.

Dye Reagent II: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use. Keep in dark and store at 4 °C for 6 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 12,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For Cell culture media and other biological fluids samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents intothe microcentrifuge tube:

Reagent*	Sample**	Standard	Blank
Sample	20µl	--	--
Distilled water	--	--	40 µl
Standard	--	40 µl	--
Substrate	20 µl	--	--
Mix,incubate at room temperaturefor 15 minutes.			
Stop Solution	50 µl	50 µl	50 µl
Centrifuged at 10,000g for 10 minutes, then transfer the supernatant into the microplate.			
Dye Reagent I	100 µl	100 µl	100 µl
Dye Reagent II	10 µl	10 µl	10 µl
Mix,incubate at room temperature for 5 minutes, record absorbance measured at 500nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit ofGlycolate Oxidase activity is the enzyme thatoxidizes 1μmol ofthe Glycolic acid per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Blank}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times C_{Protein}\times T}(U/mg)$$

2.2 According to the weight of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample}/ V_{Assay})\times T}(U/g)$$

2.3 According to the volume of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

$C_{Standard}$: the concentration of standard, mmol/L= μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

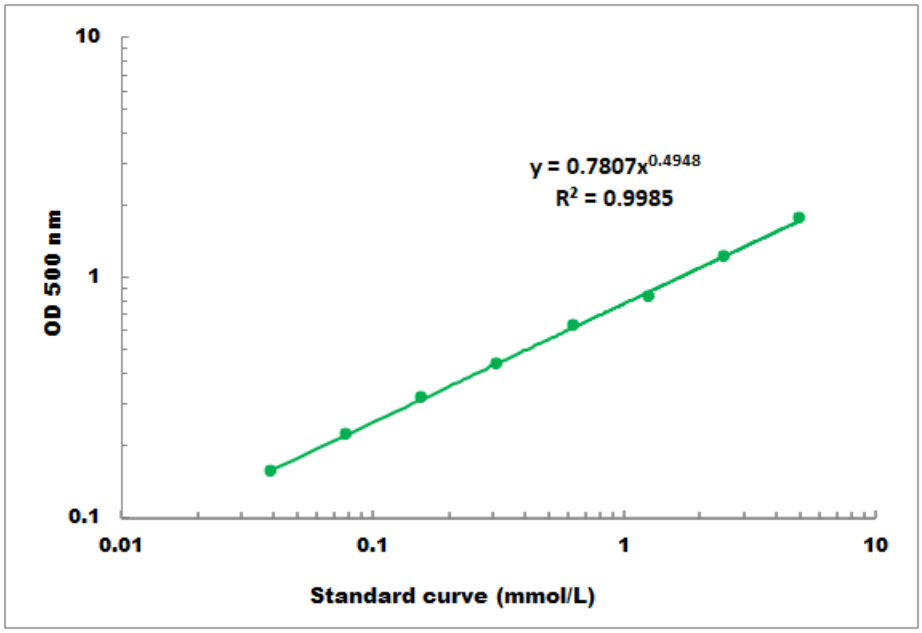
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05mmol/L - 5mmol/L