Glycolate Oxidase

Microplate Assay Kit

User Manual

Catalog # CAK1103

(Version 1.4C)

Detection and Quantification of Glycolate Oxidase (GOX) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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.

The assay is initiated with the enzymatic oxidization of the Glycolic acid by Glycolate oxidase. The enzyme catalysed reaction product Glyoxylic acid react with Phenylhydrazine, glyoxylate phenylhydrazone can be measured at a colorimetric readout at 500 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Dye Reagent I	Powder x 1	4 °C, keep in dark
Dye Reagent II	Powder x 1	4 °C, keep in dark
Dye Reagent I Diluent	10 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Stop Solution	5 ml x 1	4 °C
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Note:

Substrate: add 2 ml distilled water to dissolve before use, store at 4 °C.

Standard: add 1 ml distilled water to dissolve before use; then add 0.1 ml into 0.9 ml distilled water, mix; the concentration will be 5 mmol/L, store at 4 °C.

Dye Reagent I: add 10 ml Dye Reagent I Diluent to dissolve before use, store at 4 °C. If the color change to yellow, it may be out of work.

Dye Reagent II: add 1 ml distilled water to dissolve before use, store at 4 °C.

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. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tube:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Distilled water	--	--	40 µl
Standard	--	40 µl	--
Substrate	20 µl	--	--
Mix, incubate at room temperature for 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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Glycolate Oxidase activity is the enzyme that oxidizes 1 μmol of the Glycolic acid per minute.

1. According to the protein concentration of sample

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

$$= 0.667 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.667 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$GOX\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.667 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of Standard, 5 mmol/L = 5 μmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

V_{Sample}: the volume of sample, 0.02 ml;

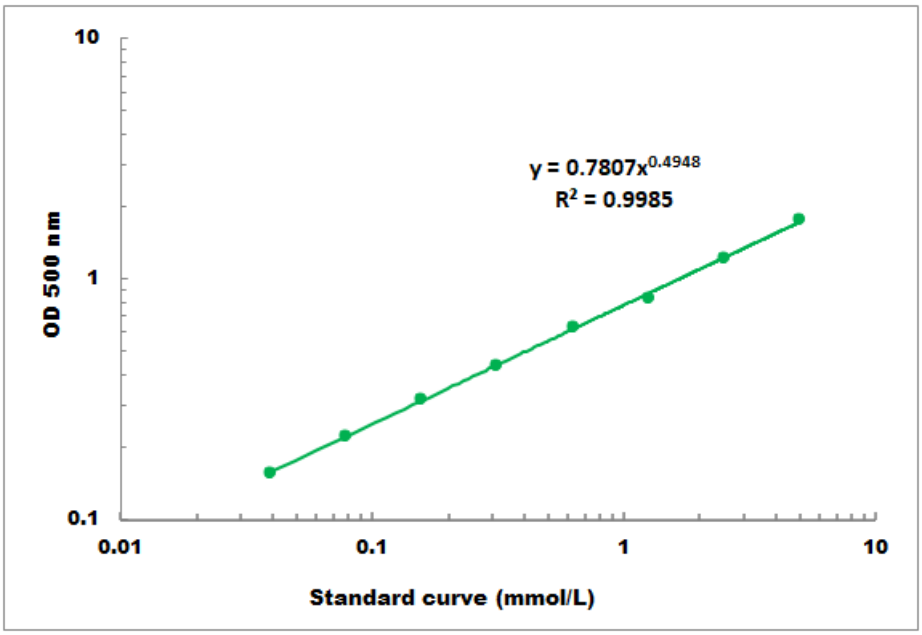
$V_{Standard}$: the volume of standard, 0.04 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 15 minutes.

VII. TYPICAL DATA

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



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES