Glycolate Oxidase Microplate Assay Kit

Catalog # AS0103

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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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 byGlycolate oxidase. The enzyme catalysed reaction product Glyoxylic acid react 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	

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 mlDye 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
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. 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.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	20µl	--	--
Distilled water	--	--	40 µl
Standard	--	40 µl	--
Substrate	20 µl	--	--
Dye Reagent I	100 µl	100 µl	100 µl
Dye Reagent II	10 µl	10 µl	10 µl
Mix,incubate at room temperaturefor 15 minutes.			
Stop Solution	50 µl	50 µl	50 µl
Mix,record absorbance measured at 500nm.			

VI. CALCULATION

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

1. According to the protein concentration of sample

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

$= 0.167 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

2. According to the weight of sample

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

$= 0.167 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the volume of sample

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

$= 0.167 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

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

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

W: the weight of sample, g;

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

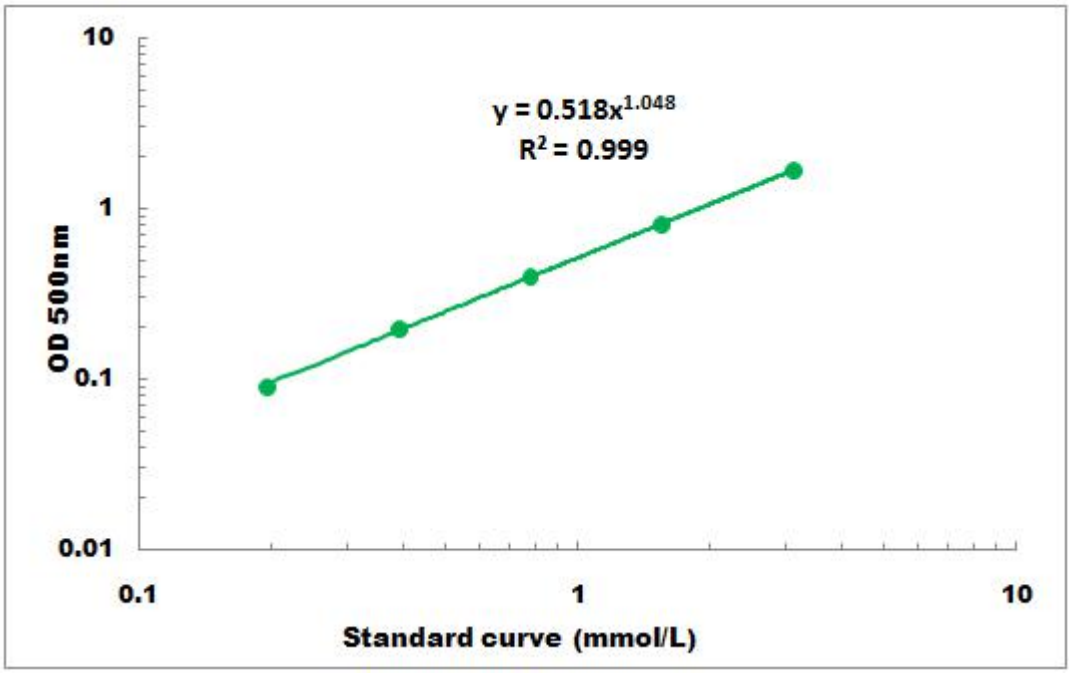
$V_{Standard}$: the volume of standard, 0.04 ml;

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: 0.1mmol/L - 5mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES