Glycolate Oxidase Assay Kit

Glycolate Oxidase Assay Kit is a detection kit for the quantification of Glycolate Oxidase Activity in tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82029

Package: 96 wells

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INTRODUCTION

This gene is one of three related genes that have 2-hydroxyacid oxidase activity yet differ in encoded protein amino acid sequence, tissue expression and substrate preference. Subcellular location of the encoded protein is the peroxisome. Specifically, this gene is expressed primarily in liver and pancreas and the encoded protein is most active on glycolate, a two-carbon substrate. Glycolate oxidase oxidizes glycolic acid to glyoxylate, and can also oxidize glyoxylate into oxalate. These reactions are central to the toxicity of ethylene glycol poisoning.

The protein is also active on 2-hydroxy fatty acids. The transcript detected at high levels in pancreas may represent an alternatively spliced form or the use of a multiple near-consensus upstream polyadenylation site. [Provide by Wikipedia: Glycolate oxidase]

PRINCIPLE OF THE ASSAY

This glycolate oxidase Assay Kit is a simple colorimetric assay that measures the amount of glycolate oxidase present in tissue extracts, cell lysate and cell culture supernatants. The assay is based on the enzyme driven reaction. 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. Samples and standards are read with a plate reader. The concentration of glycolate oxidase in the samples is then determined by comparing the O.D. 500 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Microplate	1 X 96-well plate	RT
Standard	1 vial (lyophilized)	4°C
Assay Buffer	4 x 30 mL (ready to use)	4°C
Substrate	1 vial (lyophilized)	4°C
Dye Reagent A	1 vial (lyophilized)	4°C (protect from light)
Dye Reagent B	1 vial (lyophilized)	4°C (protect from light)
Dye Reagent A Diluent	10 mL (ready to use)	4°C
Stop Solution	5 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading in the 500 nm range
- Centrifuge
- Mortar
- Deionized or Distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Dye Reagent should be store at 4°C and protect from light.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes briefly and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL Assay Buffer on ice. Centrifuge samples 12,000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Cell culture supernatant and other biological fluids samples: To remove insoluble particles, centrifuge samples 1,000- X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Substrate:** Reconstitute the Substrate with **2 ml** of **distilled water.** Make sure the Substrate is dissolved completely and mixed thoroughly before use. The reconstituted Substrate can be stored at 4°C for up to a week.
- **Dye Reagent A:** Reconstitute the Dye Reagent A with **10 ml** of **Dye Reagent A Diluent**. Make sure the reagent is dissolved completely and mixed thoroughly before use. The reconstituted Dye Reagent A can be stored at 4°C for up to a week.
- **Dye Reagent B:** Reconstitute the Dye Reagent B with **1 ml** of **distilled water**. Make sure the reagent is dissolved completely and mixed thoroughly before use. The reconstituted Dye Reagent B can be stored at 4°C for up to a week.
- **Standards:** Reconstitute the Standard with **1 ml** of **distilled water** to yield a stock concentration of 50 µmol/ml (50 mmol/L). Make sure the Stock Standard is dissolved completely and mixed thoroughly before use. Add **0.1 ml** of the **stock standard** into **0.9 ml** of **distilled water** to yield a working standard at concentration of **5 µmol/ml (5 mmol/L).** The reconstituted standard stock can be aliquoted and stored at 4°C for up to a week.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with Assay buffer or PBS before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Each Standard and sample should be assayed in duplicate or triplicate.

1. Add **20 µL** of each **Sample** in Sample wells of the 96-well microplate.
2. Add **40 µL** of **Distilled water** in Blank well of the 96-well microplate
3. Add **40 µL** of diluted **Standard** in Standard well of 96-well microplate.
4. Add **20 µL** of **Substrate** into Sample wells.
5. Mix well and incubate plate for **15 min** at **RT** in the dark.
6. Add **50 µL** of **Stop Solution** into each well, mix thoroughly.
7. Centrifuged at 10,000g for **10 minutes**, then transfer the supernatant into the microplate.
8. Add **100 µL** of **Dye Reagent A** into each well.
9. Add **10 µL** of **Dye Reagent B** into each well.
10. Mix well and incubate plate for **5 min** at **RT**.
11. Read the plate with a microplate reader at **500 nm**.

Summary of Glycolate Oxidase Assay Procedure

Reagent	Sample	Standard	Blank
Sample	20 µl	-	-
Distilled water	-	-	40 µl
Standard	-	40 µl	-
Substrate	20 µl	-	-
Mix, incubate plate for **15 min** at **RT** in the dark.			
Stop Solution	50 µl	50 µl	50 µl
Centrifuged at 10,000g for **10 minutes**, then transfer the supernatant into the microplate.			
Dye Reagent A	100 µl	100 µl	100 µl
Dye Reagent B	10 µl	10 µl	10 µl

Mix incubate at **RT** for **5 mins**. Read the OD with a microplate reader at **500 nm** immediately.

CALCULATION OF RESULTS

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

2. Calculate the average absorbance values for each set of samples, standard and blank.

3. Calculation:

A. Definition:

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

W: the weight of sample, g;

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

$V_{Standard}$: the volume of the standard, 40 µl = 0.04 ml;

V_{Sample}: he volume of reaction sample, 20 µl = 0.02 ml;

V_{total}: the total volume of Assay buffer for tissue sample, 1 ml;

T: the reaction time, 15 minutes.

B. Formula:

a). According to the protein concentration of sample

Glycolate Oxidase activity (U/mg) =

$[(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times C_{Protein}) \times T]$

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

b). According to the weight of sample

Glycolate Oxidase activity (U/g) =

$[(C_{Standard} \text{ X } V_{Standard}) \text{ X } (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \text{ X } (W \times V_{Sample} / V_{total}) \text{ X } T]$

$= 0.667 \text{ X } (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \text{ X } W]$

c). According to the volume of sample

Glycogen Branching Enzyme activity (U/ml) =

$[(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \text{ X } V_{Sample} \text{ X } T]$

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

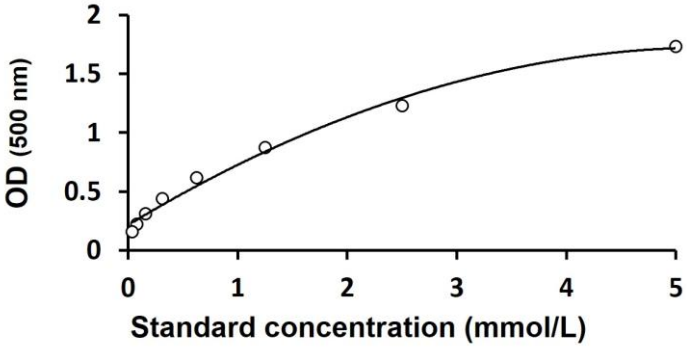
4. Detection range:

The detection range is from 0.05 mmol/L - 5 mmol/L.

5. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and this kit does not need serial diluted standards.



QUALITY ASSURANCE

Sensitivity

0.05 mmol/L