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Product Information & Manual

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L–α–Glycerophosphate Oxidase (GPO)

Cat no. LDG0037RG

Product Overview

Description

Glycerol-3-phosphate Oxidase (GPO) is a flavin-dependent enzyme that catalyzes the oxidation of sn-glycerol-3-phosphate to dihydroxyacetone phosphate (DHAP), with the production of hydrogen peroxide (H_2O_2) as a byproduct. This reaction plays an essential role in glycerol metabolism, serving as a critical link between lipid catabolism, glycolysis, and lipid biosynthesis through the generation of DHAP—a central intermediate in energy and metabolic pathways. In clinical and biochemical applications, GPO is frequently used in enzymatic assays for the quantification of triglycerides and glycerol. The hydrogen peroxide produced is coupled with a colorimetric reaction, allowing for easy detection and measurement. Due to its specificity and efficiency, GPO plays a vital role in analytical biochemistry and is a useful tool in both clinical laboratories and metabolic research.

Expression system

Escherichia coli

Specification

Appearance	Yellow powder, lyophilized
Activity	10 U/mg or more

Properties

Stability	Stable at –20℃ for at least one year
Isoelectric point	5.09

Reconstitution

It is recommended to weigh and reconstitute 10 mg of lyophilized powder in 125 µL double-distilled water directly and incubate the solution for at least 10 mins to ensure sufficient re-dissolved.

Assay

1. Assay principle

L-α-Glycerol-phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dihydroxyacetone phosphate + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye+ $4H_2O$

2. Unit definition

One unit is defined as the enzyme quantity which oxidizes one micromole of L-α-Glycerol-phosphate per minute under the following conditions: 84 mM Potassium phosphate (pH 7.0), 425 mM L-α-Glycerol-phosphate, 0.03% 4-Aminoantipyrine, 0.024% Phenol, and 1.9 U/mL Peroxidase.

3. Reagents

A. Phosphate buffer, pH 7.0	0.2 M (KH_2PO_4-NaOH)
B. 4-Aminoantipyrine solution	0.5% (w/v) (Dissolve in deionized water)
C. Phenol solution	5% (w/v) (Dissolve in deionized water)

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D. Substrate solution	Weigh and dissolve 1.835 g of disodium (+/-)-1-glycerophosphate n-hydrate in approximately 8 mL of deionized water. Adjust the pH to 7.0 with 3 N HCl and fill up to 10 mL with deionized water. Then, filtrate the solution with 0.45 µm membrane filter.
E. Peroxidase solution	200 U/mL [Dissolve in 0.2 M Phosphate buffer (A)]
F. Aminoantipyrine-phenol solution	Mix approximately 6 mL of 0.2 M Phosphate buffer (A), 0.2 mL of Peroxidase solution (E), 1.2 mL of 4-Aminoantipyrine solution (B) and 0.1 mL of Phenol solution (C), then fill up to 10 mL with 0.2 M Phosphate buffer (A).
G. Enzyme diluent	Weigh 0.66 g of ammonium sulfate and dissolved in 1 mL of 0.2 M Phosphate buffer (A). Add and dissolve 10 mg of bovine serum albumin, then fill up to 10 mL with deionized water.

4. Procedure

(1) Prepare the following **Working Solution** immediately before use and equilibrate at 37℃ for approximately 5 minutes (for 4 reactions).

Working Solution

Aminoantipyrine-phenol solution **(Reagent F)**	3 mL
Substrate solution **(Reagent D)**	3 mL
Total	**6 mL**

(2) Pipette 1.5 mL of **Working Solution** into a tube.

(3) Add 0.05 mL of the enzyme solution* and mix by gentle inversion.

Concentration in a reaction	
Phosphate buffer (KH_2PO_4-NaOH)	84 mM
4-Aminoantipyrine	0.03%
Phenol	0.024%
Peroxidase	1.9 U/mL
Disodium (+/-)-1-glycerophosphate n-hydrate	425mM

(4) Pipette the mixture into a cuvette (d=1.0 cm). Record the increase in optical density at 505 nm against water for 1 to 5 minutes in a spectrophotometer at room temperature and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test). At the same time, measure the blank rate (ΔOD blank) by using the same method as the test except that the enzyme diluent is added instead of enzyme solution.

* Dissolve the enzyme preparation in ice-cold enzyme diluent **(Reagent G)** dilute to **0.07–0.11 U/mL** with the same buffer and store on ice.

(5) Activity can be calculated by using the following formula:

Volume activity (U/mL) =

$$\frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times Vt \times 2 \times df}{\frac{13.2 \times 1.0 \times Vs}{}}$$

$$= \Delta OD/min \times 4.696 \times df$$

Weight activity (U/mg) = (U/mL)×1/C

Vt: Total volume (1.55 mL)

Vs: Sample volume (0.05 mL)

13.2: Millimolar absorption coefficient of quinoneimine dye

2: Conversion factor (one mole of quinoneimine dye corresponds to two moles of glycerophophate)

1.0: Light path length (cm)

df: Dilution factor

C: Enzyme concentration in dissolution (mg/mL)

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The effect of different conditions on Glycerol-3-phosphate Oxidase

A.

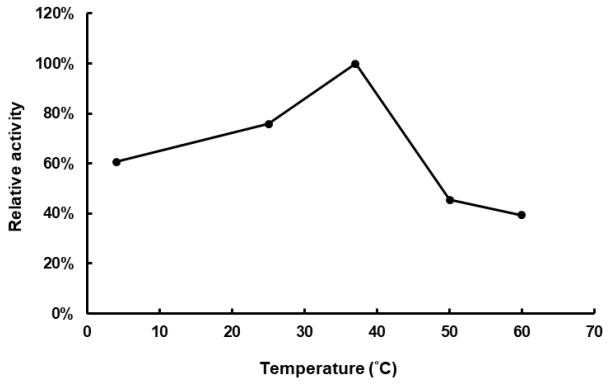

Figure A. Temperature activity of GPO. The enzyme reactions in 0.2 M KH_2PO_4-NaOH, pH 7.0, were carried out under different temperatures.

B.

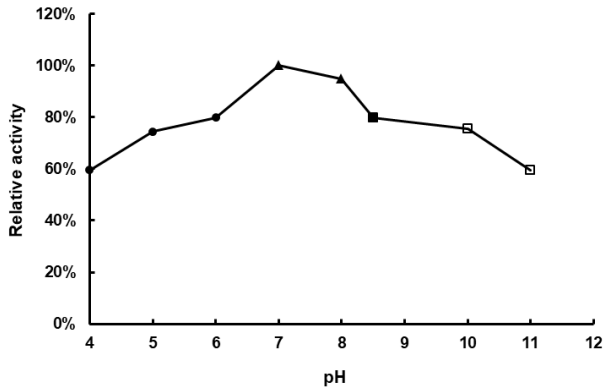

Figure B. pH activity of GPO. The buffer conditions with various pH values were used in the reaction at 37°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

C.

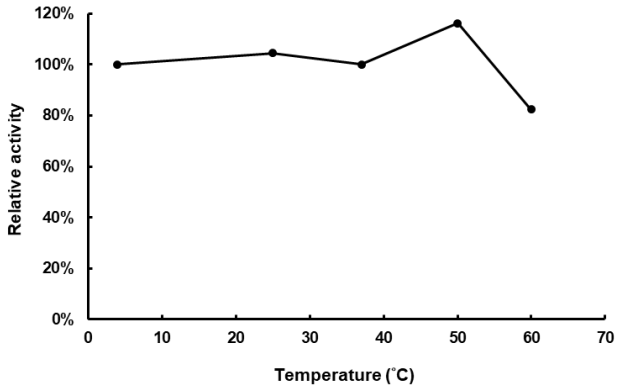

Figure C. Thermal stability of GPO. The enzyme powder was reconstituted by double-distilled water and treated with different temperatures for 15 minutes. Final concentration: 21.6 U/mL

D.

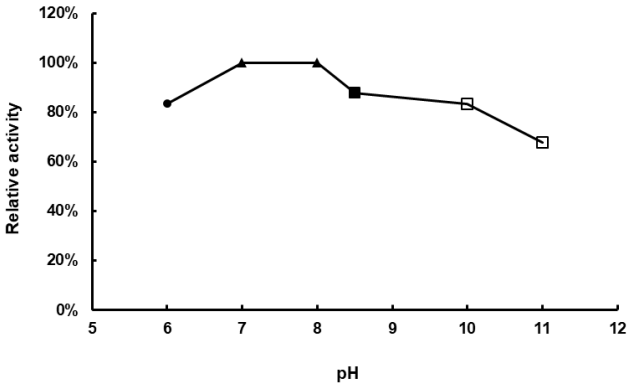

Figure D. pH stability of GPO. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer condition for 20 hours. pH 6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

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E.

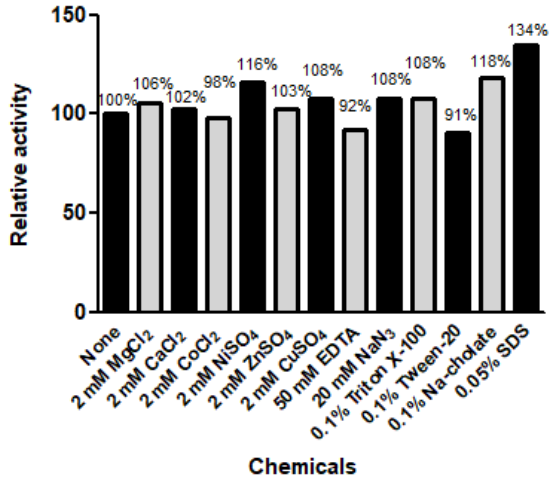

Figure E. The effects of various chemicals on GPO.
The enzyme was incubated with the chemicals at 25°C for 1 hour.

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For Research Use or Further Manufacturing Only.

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