GLYCEROL KINASE [GKⅢ]

from Microorganism
(ATP:Glycerol 3-phosphotransferase, EC 2.7.1.30)

Glycerol + ATP ⟶ *sn*-Glycerol 3-phosphate + ADP

Preparation and Specification

Appearance	: White to brownish lyophilized powder
Specific activity	: More than 50 U/mg solid
Contaminants	: Catalase Less than 0.1%(U/U)

Properties

Molecular weight	: 50 kDa (SDS-PAGE)	
Isoelectric point	: 4.73 (estimated from amino acid sequence)	
Michaelis constants	: Glycerol 5.3×10^{-5}M	
	ATP 4.8×10^{-4}M	
Optimum pH	: 6.5-7.0	Figure 1
pH stability	: 5.5–9.0 (37℃, 60 min)	Figure 2
Thermal stability	: Stable at 50℃ and below (pH7.0, 15 min)	Figure 3
Optimum temperature	: 45℃	Figure 4
ProClin stability	: See Figure 5	

Preparation and Specification

This enzyme is useful for enzymatic determination of **triglyceride (TG)** coupled with LP (Lipase; T-01, T-63, or T-116) and GPO (L-α-Glycerophosphate oxidase; T-60 or T-107).

$$\text{TG} + 3\,H_2O \xrightarrow{\text{LP}} \text{Glycerol} + 3\ \text{Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GKⅢ}} \text{G-3-P} + \text{ADP}$$

$$\text{G-3-P} + O_2 \xrightarrow{\text{GPO}} \text{DHAP} + H_2O_2$$

$$2\,H_2O_2 + 4\text{-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinonimine dye} + 4\,H_2O$$

G-3-P: *sn*-Glycerol 3-phosphate
DHAP: Dihydroxyacetonephosphate

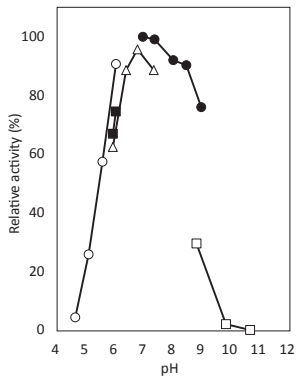

Fig. 1 Optimum pH
○: Acetate buffer
■: Phosphate buffer
△: PIPES-NaOH buffer
●: Tris-HCl buffer
□: Glycine-NaOH buffer

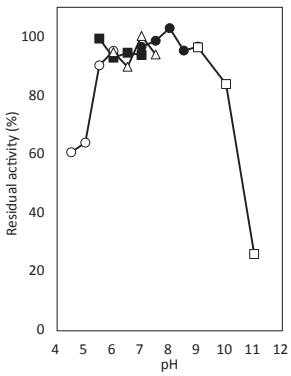

Fig. 2 pH stability
37°C, 60 min
○: Acetate buffer
■: Phosphate buffer
△: PIPES-NaOH buffer
●: Tris-HCl buffer
□: Glycine-NaOH buffer

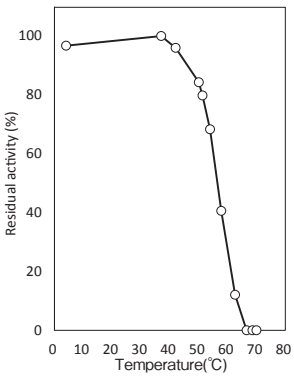

Fig. 3 Thermal stability
pH7.0, 15 min
50 mM Phosphate buffer

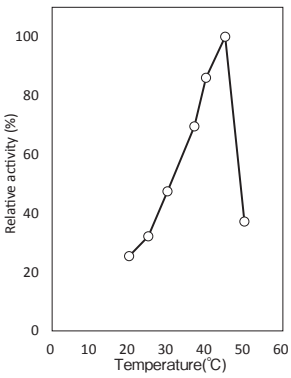

Fig. 4 Optimum temperature
pH7.0
100 mM PIPES-NaOH buffer

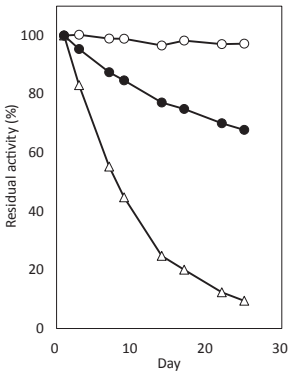

Fig. 5 ProClin stability
20°C, pH7.0
50 mM PIPES-NaOH + 0.5% ProClin
○: GK III (T-223)
●: Competitor A
△: Competitor B

Assay

■ Principle

The assay is based on the increase in absorbance at 546 nm as the formation of quinoneimine dye in the following reactions:

$$\text{Glycerol + ATP} \xrightarrow{\text{GK III}} \text{Glycerol 3-Phosphate + ADP}$$

$$\text{Glycerol 3-Phosphate + } O_2 \xrightarrow{\text{GPOSP}} \text{Dihydroxyacetonephosphate + } H_2O_2$$

$$2H_2O_2 + \text{4-AA + TODB} \xrightarrow{\text{POD}} \text{Quinoneimine dye + } 4H_2O$$

GPOSP: L-α-Glycerophosphate oxidase
POD: Peroxidase

■ Unit definition

One unit is defined as the amount of enzyme which converts 1 μmole of glycerol to glycerol 3-phosphate per minute at 37° C under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture

0.5 M PIPES-NaOH buffer pH7.0	0.2 ml
5% Glycerol solution	0.05 ml
100 mM ATP solution pH7.0	0.1 ml
100 mM $MgCl_2$ solution	0.1 ml
100 U/ml GPOSP solution	0.1 ml
100 U/ml POD solution	0.05 ml
0.2% TODB solution	0.1 ml
0.3% 4-AA solution	0.1 ml
Distilled water	0.2 ml

2. Reaction stopper
   0.5% SDS solution
3. Enzyme dilution buffer
   50 mM PIPES-NaOH buffer pH 7.0 containing 0.1%(W/V) BSA.
4. Reagents
   PIPES [Piparazine-1,4-bis(2-ethanesulphonic acid)]:
   Dojindo Laboratories #345–02225

Glycerol: FUJIFILM Wako Pure Chemical Corporation Guaranteed Reagent #075-00616
ATP ($2Na \cdot 3H_2O$): Kyowa Hakko Co., Ltd.
$MgCl_2 \cdot 6H_2O$: FUJIFILM Wako Pure Chemical Corporation Guaranteed Reagent #131-00162
GPOSP: Asahi Kasei Pharma Corporation #T-60
TODB (N,N-Bis(4-sulfobutyl)-3-methylaniline, disodium salt): Dojindo Laboratories #OC22
4-AA: NACALAI TESQUE, INC. Guaranteed Reagent #01907–52
POD: Sigma Chemical Co. Type II #P-8250
BSA: Millipore Fraction V pH 5.2 #81–053
SDS (Sodium Dodecyl Sulfate): NACALAI TESQUE, INC. #31606

■ Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette accurately 1.0 ml of reaction mixture into a small test tube and preincubate at 37° C.
2. After 5 min, add 40 μl of enzyme solution and mix to start the reaction at 37° C.

※ In the case of a test blank, add 40 μl of enzyme dilution buffer in place of enzyme solution.

3. At 5 min after starting the reaction, add 2.0 ml of the reaction stopper to stop the reaction.
4. Measure the absorbance at 546 nm.

Absorbance sample : As
blank : Ab
△ A = (As − Ab) ≦ 0.6 Abs

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/5}{39.2 \times 1/2} \times \frac{3.04}{0.04} \times \frac{1}{X}$$

39.2 : millimolar extinction coefficient of quinoneimine dye at 546 nm ($cm^2/\mu mol$)
1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produce 1 mole of quinoneimine dye
5 : reaction time (min)
3.04 : final volume (ml)
0.04 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at -20° C in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition.

GKⅢ活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液

0.5M PIPES-NaOH 緩衝液 pH 7.0	0.2 ml
5% グリセロール液	0.05 ml
100mM ATP 溶液 pH7.0	0.1 ml
100mM 塩化マグネシウム溶液	0.1 ml
100U/ml GPOSP	0.1 ml
100U/ml POD	0.05 ml
0.2% TODB	0.1 ml
0.3% 4-AA	0.1 ml
精製水	0.2 ml

2. 反応停止液
   0.5% SDS 溶液
3. 酵素溶解希釈用液
   0.1%（W/V）BSA を含む 50 mM PIPES-NaOH 緩衝液 pH7.0
4. 試薬
   PIPES（ピペラジン -N,N'- ビス（2- エタンスルフォン酸））: 同仁化学製 #345-02225
   グリセロール : 富士フイルム和光純薬製 特級 #075-00616
   ATP（アデノシン三リン酸・$2Na \cdot 3H_2O$）: 協和発酵製
   塩化マグネシウム : 富士フイルム和光純薬製 特級 #131-00162
   GPOSP: 旭化成ファーマ製 #T-60
   TODB（N,N-Bis（4-sulfobutyl）-3-methylaniline, disodium salt）: 同仁化学製 #OC22
   4-AA（4- アモノアンチピリン）: ナカライテスク製 特級 #01907–52
   POD: シグマ製 Type II #P-8250
   BSA: ミリポア製 Fraction V pH 5.2 #81–053
   SDS（ドデシル硫酸ナトリウム）: ナカライテスク製 #31606

Ⅱ．酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液で溶解して全容 20ml とする。その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ．測定操作法

1. 小試験管に反応試薬混合液 1.0ml を正確に分注して 37℃で予備加温する。
2. 5 分経過後、酵素試料液 40 μl を正確に加えて混和し、37℃で反応を開始する。
   ※盲検は酵素試料液の代わりに酵素溶解希釈用液 40 μl を加える。
3. 5 分経過後、反応停止液 2.0ml を加えて混和し、反応

を停止する。

4. 546nm における吸光度を測定する。求められた吸光度を試料液については As、盲検液については Ab とする。

※吸光度範囲　$\Delta A = (As - Ab) \leqq 0.6$ Abs

Ⅳ. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/5}{39.2 \times 1/2} \times \frac{3.04}{0.04} \times \frac{1}{X}$$

39.2 : キノンイミン色素の 546nm におけるミリモル分子吸光係数 ($cm^2/\mu mol$)

1/2 : H_2O_2 2 モルからキノンイミン色素 1 モルが生成することによる係数

5 : 反応時間 (min)

3.04 : 反応総液量 (ml)

0.04 : 反応に供した酵素試料液量 (ml)

X : 酵素試料液中の検品濃度 (mg/ml)