

(Diagnostic Reagent Grade)

T-223

GLYCEROL KINASE [GK III]

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



Preparation and Specification

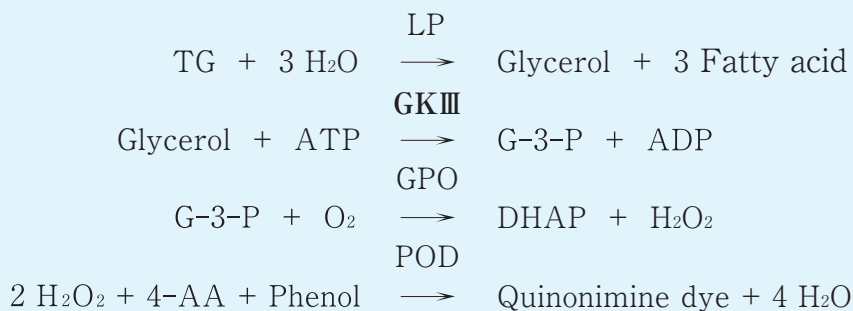
Appearance : White to brownish lyophilized powder
Specific activity : More than 50 U/mg solid
Contaminants : Catalase Less than 0.1%(U/U)

Properties

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



G-3-P: sn-Glycerol 3-phosphate

DHAP: Dihydroxyacetone phosphate

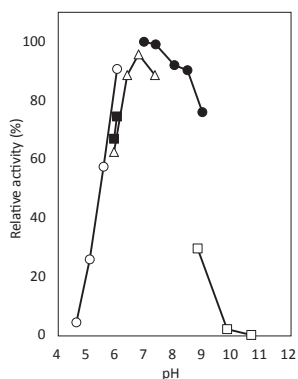


Fig. 1 Optimum pH

○: Acetate buffer
■: Phosphate buffer
△: PIPES-NaOH buffer
●: Tris-HCl buffer
□: Glycine-NaOH buffer

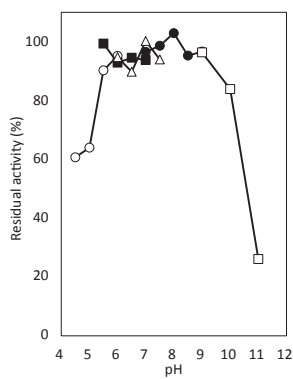


Fig. 2 pH stability

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

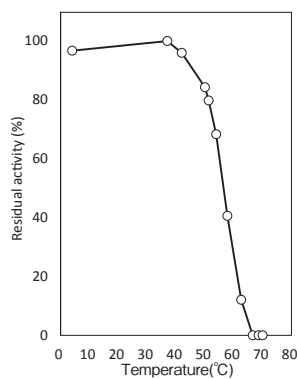


Fig. 3 Thermal stability

pH7.0, 15 min
50 mM Phosphate buffer

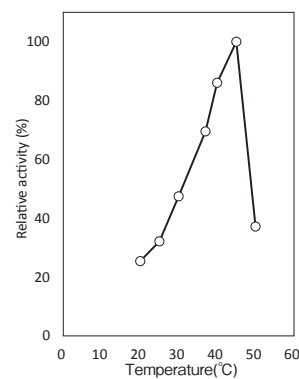


Fig. 4 Optimum temperature

pH7.0
100 mM PIPES-NaOH buffer

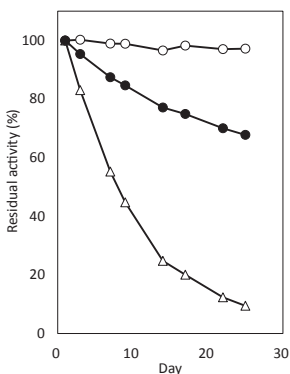


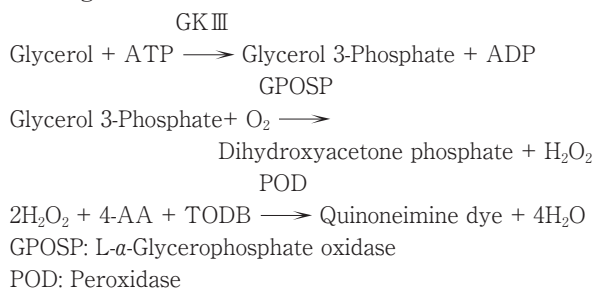
Fig. 5 ProClin stability

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:



■ Unit definition

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

■ Reagents

- 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 GPOS 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
- Reaction stopper

0.5% SDS solution	
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- Enzyme dilution buffer

50 mM PIPES-NaOH buffer pH 7.0 containing 0.1%(W/V) BSA.	
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- 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·3H₂O): Kyowa Hakko Co., Ltd.
MgCl₂·6H₂O:

FUJIFILM Wako Pure Chemical Corporation
Guaranteed Reagent #131-00162

GPOSP: Nagase Diagnostics Co., Ltd. #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

$$\Delta A = (A_s - A_b) \leq 0.6 \text{ Abs}$$

■ Calculation

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

39.2 : millimolar extinction coefficient of quinoneimine dye at 546 nm (cm²/μmol)

1/2 : a multiplier derived from the fact that 2 mole of H₂O₂ 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-エタンスルホン酸)): 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·3H₂O):

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: ナガセダイアグノスティックス製 #T-60

TODB (N,N-Bis(4-sulfobutyl)-3-methylaniline, disodium salt): 同仁化学製 #OC22

4-AA (4-アミノアンチピリン):

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°C 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°C 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 A_s 、盲検液については A_b とする。

※吸光度範囲 $\Delta A = (A_s - A_b) \leq 0.6 \text{ Abs}$

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 ($\text{cm}^2/\mu\text{mol}$)

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)