GLYCEROL KINASE [GKZ]

from *Flavobacterium meningosepticum*
(ATP: Glycerol–3–phosphotransferase, EC 2.7.1.30)

Glycerol + ATP ⟶ L– α –Glycerolphosphate + ADP

★ Advantages
① Thermal stability
② Storage stability
③ Antiseptic stability

Preparation and Specification

Appearance : White to light grayish white amorphous powder, lyophilized
Specific activity : More than 70 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 150 kDa (TSK G3000SWXL) 50 kDa (SDS–PAGE)	
Isoelectric point	: pH 4.3	
Michaelis constants	: Glycerol 8.8×10^{-5}M ATP 3.0×10^{-5}M	
Optimum pH	: 8.0	Figure 1
pH stability	: 5.0–11.0 (37℃, 60 min)	Figure 2
Optimum temperature	: 80℃ (Tris–HCl buffer)	Figure 3
Thermal stability	: Stable at 60℃ and below (pH 6.5, 10 min) Comparison data between GKZ and GK	Figure 4
Antiseptic stability	: See Figure 5	
Reactivity after long incubation	: See Figure 6	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **triglyceride**.

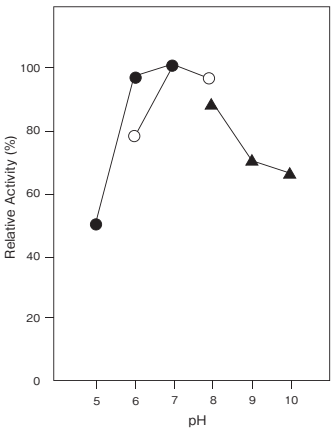
$$TG + 3\,H_2O \xrightarrow{LPBP} \text{Glycerol} + 3\ \text{Fatty acid}$$
$$\text{Glycerol} + ATP \xrightarrow{\textbf{GKZ}} G-3-P + ADP$$
$$G-3-P + O_2 \xrightarrow{GPOSP} DHAP + H_2O_2$$
$$2\,H_2O_2 + 4\text{–AA} + \text{Phenol} \xrightarrow{POD} \text{Quinoneimine dye} + 4\,H_2O$$

TG: Triglyceride, DHAP: Dihydroxyacetonephosphate

Table 1. Substrate Specificity

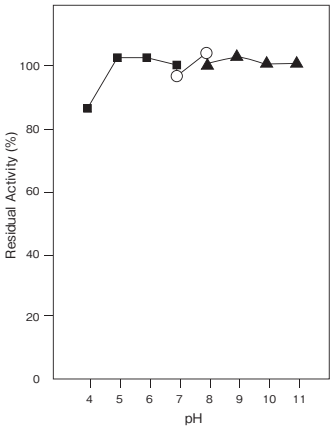
Substrate	Relative activity (%)
Glycerol	100
Glycerol-α-monochlorohydrin	0
Ethylene glycol	0
1,3-Propanediol	0
1,3-Butanediol	0
1,4-Butanediol	0
1,2-Butanediol	0
d-Mannitol	0
d-Sorbitol	0
d-Glucose	0
Robitol	0

Fig.1 pH Optimum



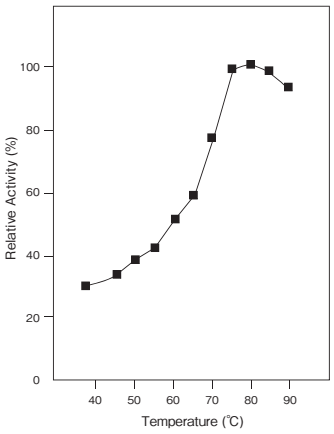
● : Phosphate buffer
○ : Tris-HCl buffer
▲ : Glycine-NaOH buffer

Fig.2 pH Stability



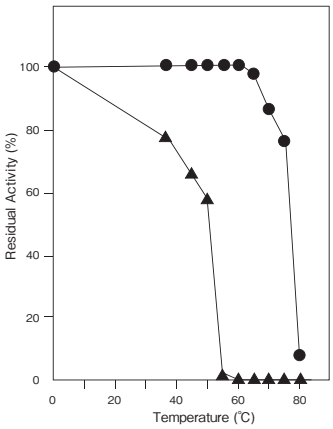
■ : 3,3-Dimethyglutarate-NaOH buffer
○ : Tris-HCl buffer
▲ : Glycine-NaOH buffer

Fig.3 Optimum temperature



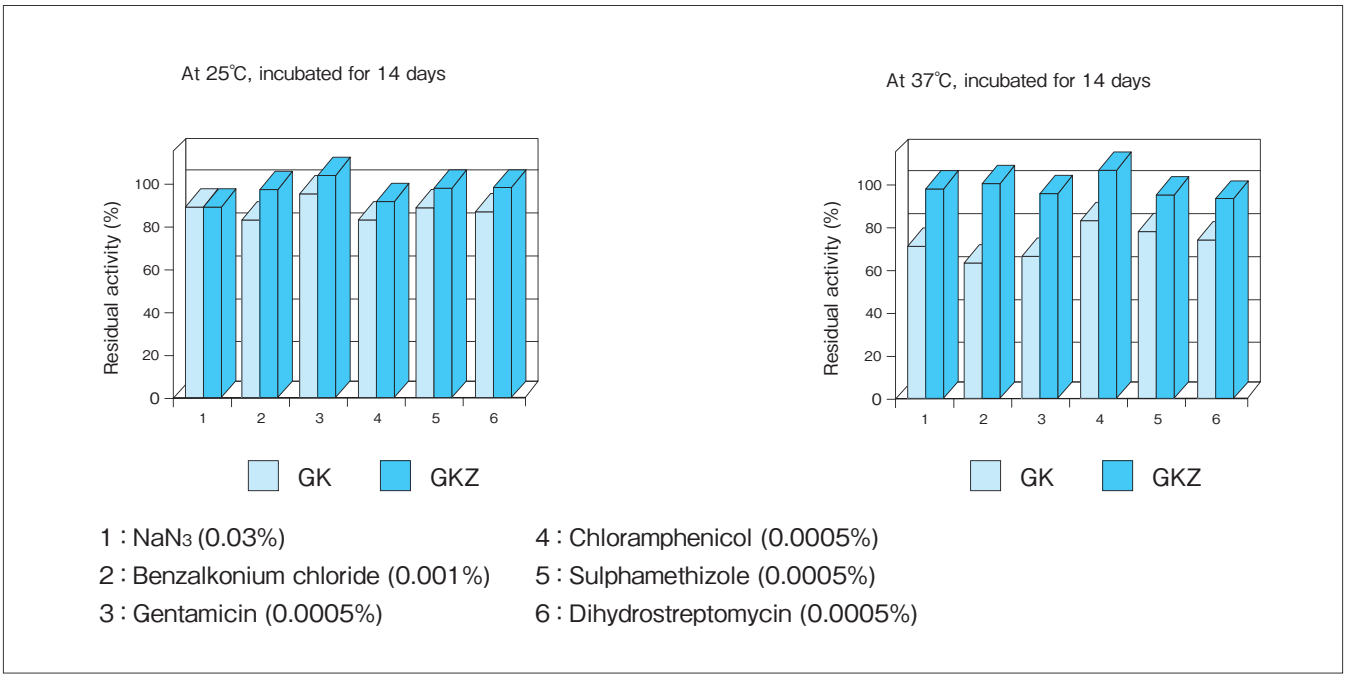
pH 8.0
100 mM Tris-HCl buffer

Fig.4 Thermal stability of GKZ



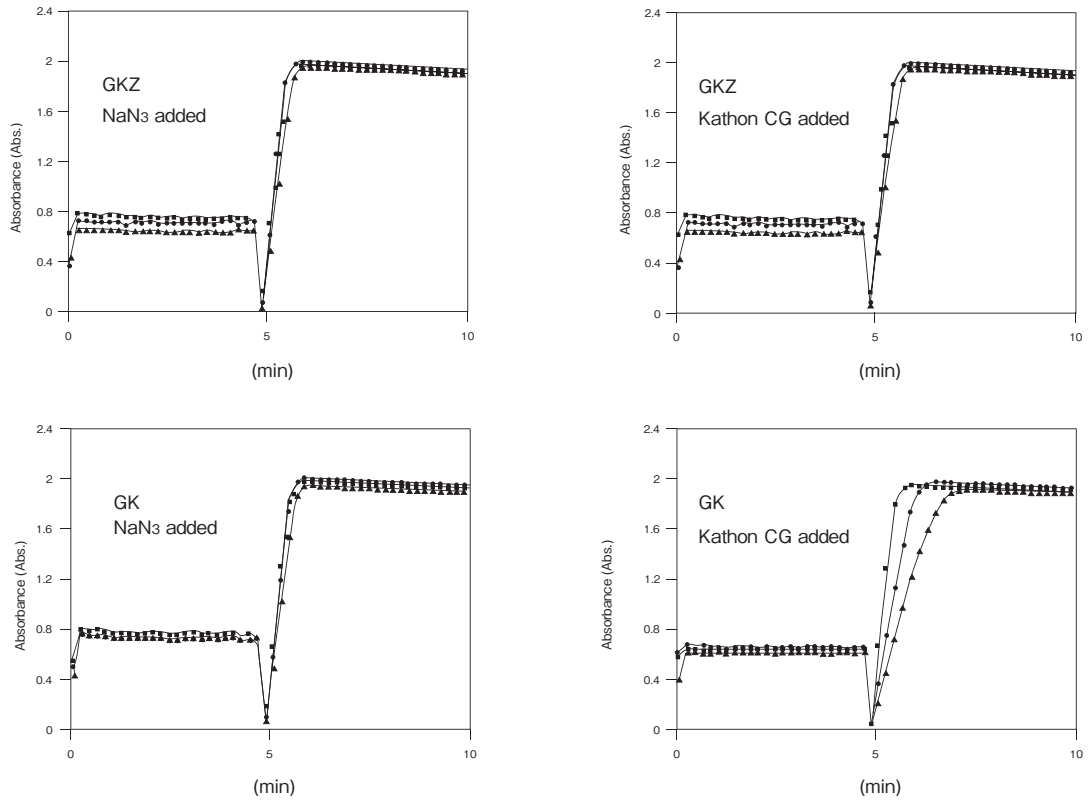
pH 6.5, 10min.
50 mM PIPES
● : GKZ(T-64)
▲ : GK

Fig.5 Resistance of GKZ against antiseptics



1 : NaN_3 (0.03%)
2 : Benzalkonium chloride (0.001%)
3 : Gentamicin (0.0005%)
4 : Chloramphenicol (0.0005%)
5 : Sulphamethizole (0.0005%)
6 : Dihydrostreptomycin (0.0005%)

Fig.6 Reactivity of GKZ after long-term incubation



Assay

■ Principle

The assay is based on the increase in absorbance at 366 nm as NADH is produced in the following reactions:

$$\text{Glycerol + ATP} \xrightarrow{\text{GKZ}} \text{Glycerol–3–P + ADP}$$

$$\text{Glycerol–3–P + NAD}^{+} \xrightarrow{\text{G3PDH}} \text{Dihydroxyacetone–P + NADH + H}^{+}$$

ATP: Adenosine triphosphate
NAD: Nicotineamido adenine dinucleotide
G3PDH: Glycerol–3–phosphate dehydrogenase

■ 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℃ under the conditions specified in the assay procedure.

■ Reagents

1. Substrate–coenzyme mixture
   80 mM ATP solution pH 7.0 2.0 ml
   0.1 M NAD solution 2.0 ml
   0.1 M Glycerol solution 2.0 ml
2. Buffer solution
   Glycine–hydrazine buffer containing 1.5 mM $MgCl_2$ pH 9.8
   Dissolve 12.5 g hydrazine hydrate and 1.50 g glycine with about 80 ml distilled water. Add 1.50 ml 0.1 M $MgCl_2$ soln. and adjust pH to 9.8 with 1N HCl at 25℃. Add distilled water to make a total of 100 ml.
3. G3PDH solution
   Use G3PDH $(NH_4)_2SO_4$ suspension (undiluted)
4. Enzyme dilution buffer
   10 mM KH_2PO_4–NaOH buffer pH 7.0 containing 10 mM glycerol
5. Reagents
   ATP: Kyowa Hakko Co., Ltd.
   Hydrazine hydrate: Tokyo Kasei Kogyo Co., Ltd. Purity 80 %
   NAD: NACALAI TESQUE, INC. #24334–84
   G3PDH: Roche Diagnostics GmbH $(NH_4)_2SO_4$ suspension

■ **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 1.80 ml of buffer into a small test tube, add 0.15 ml of substrate/coenzyme mixture and 20 μl of G3PDH, mix immediately.
   After mixing, preincubate at 37℃.
2. After 5 min, add 30 μl of enzyme solution and mix to start the reaction at 37℃.
   ※ In the case of a test blank, add 30 μl of enzyme dilution buffer in place of enzyme solution.
3. After starting the reaction, measure the rate of increase per minute in absorbance at 366 nm. The rate must be measured within the linear portion of the absorbance curve.

   Absorbance sample : As/min
   blank : Ab/min
   △A/min = (As/min − Ab/min) ≦ 0.050 Abs/min

■ **Calculation**

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/\text{min}}{3.3} \times \frac{2.00}{0.03} \times \frac{1}{X}$$

3.3 : milimolar extinction coefficient of NADH at 366 nm (cm^2/ μmole)
2.00 : final volume (ml)
0.03 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at −20℃ in the presence of a desiccant is recommended.

Reference

Sakasegawa, S., Yoshioka, I., Koga, S., Takahashi, M., Matsumoto, K., Misaki, H. and Ohshima, T. (1998) Biosci, Biotechnol, Biochem., **62**, 2388–2395

GKZ 活性測定法 (Japanese)

Ⅰ. 試薬液

1. 基質、補酵素混合液

80mM ATP 溶液 pH7.0	2.0 ml
0.1M NAD 溶液	2.0 ml
0.1M グリセロール溶液	2.0 ml

2. 緩衝液 (1.5mM $MgCl_2$ を含むグリシン–ヒドラジン緩衝液　pH9.8)
   抱水ヒドラジン (純度 80%) 12.5g とグリシン 1.50g および 0.1M 塩化マグネシウム溶液 [1)] 1.50ml を精製水 80ml に溶解した後、1N HCl で pH9.8 (25℃) に調整し、精製水で全容 100ml とする。
   1): 塩化マグネシウム溶液
   塩化マグネシウム 203mg を精製水で溶解して全容 10ml とする。
   又は抱水ヒドラジン (純度 80%) 12.5g とグリシン 1.50g 及び 0.1M 塩化マグネシウム溶液[1)] 1.50ml を精製水で溶解して全容 100ml とし、pH が 9.8 ± 0.05 (25℃) であればそのまま使用する。
3. G3PDH 溶液
   ロシュ社製の G3PDH 懸濁液をそのまま使用する。
4. 酵素溶解希釈用液
   10mM グリセロールを含む 10mM KH_2PO_4–NaOH 緩衝液 pH7.0
5. 試薬
   ATP (アデノシン三リン酸・2Na・3H_2O): 協和発酵製
   抱水ヒドラジン: 東京化成製　純度 80%
   NAD (ニコチンアミドアデニンジヌクレオチド): ナカライテスク社製　#24334–84
   G3PDH (グリセロ–3–リン酸脱水素酵素): ロシュ社製　硫安懸濁液

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. 小試験管に緩衝液 1.80ml を正確に分注して、加温する直前に基質、補酵素混合液 0.15ml と G3PDH 溶液 20 μl を分注して混和した後、直ちに 37℃で予備加温する。
2. 5 分経過後、酵素試料液 30 μl を正確に加えて混和し、直ちに 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 30 μl を加える。
3. 反応開始後、366nm における吸光度を測定して直線的に反応している 1 分間当たりの吸光度変化を求める。
   求められた吸光度変化を試料液は As/min、盲検液は Ab/min とする。
   Δ A/min = (As/min − Ab/min) ≦ 0.050 Abs/min

Ⅳ. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/\text{min}}{3.3} \times \frac{2.00}{0.03} \times \frac{1}{X}$$

3.3 : NADH の 366nm におけるミリモル分子吸光係数 (cm^2/ μmole)
2.00 : 反応総液量 (ml)
0.03 : 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)