

GLYCEROL KINASE [GKZ]

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



★ Advantages

- ① Thermal stability
- ② Stability in solution
- ③ Antiseptic stability

Preparation and Specification

Appearance : White to light grayish 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 $8.8 \times 10^{-5}\text{M}$ ATP $3.0 \times 10^{-5}\text{M}$	
Optimum pH	: 8.0	Figure 1
pH stability	: 5.0–11.0 (37°C, 60 min)	Figure 2
Optimum temperature	: 80°C (Tris-HCl buffer)	Figure 3
Thermal stability	: Stable at 60°C 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**.

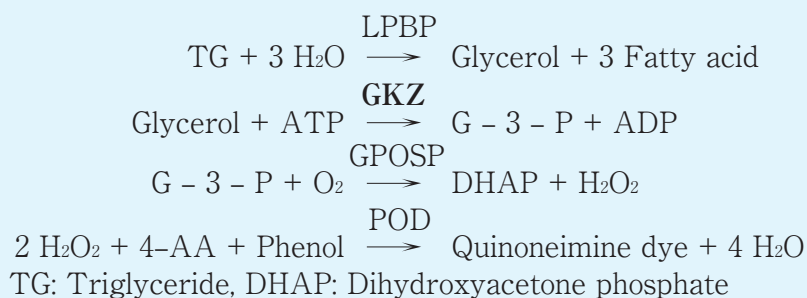


Table 1. Substrate Specificity

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

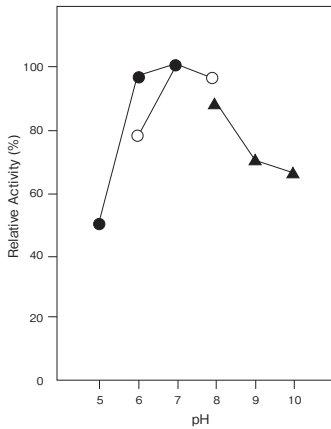


Fig.2 pH Stability

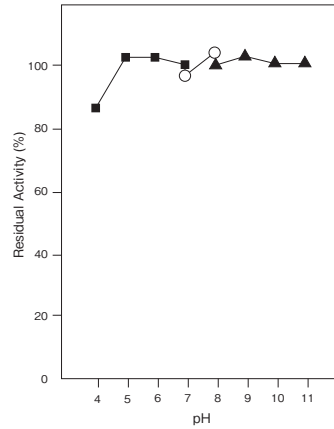


Fig.3 Optimum temperature

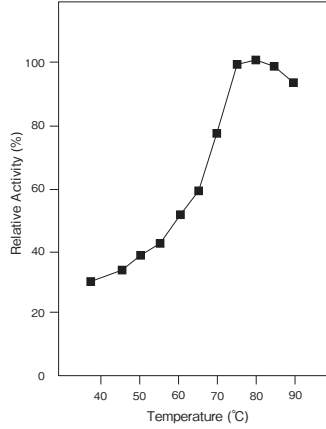
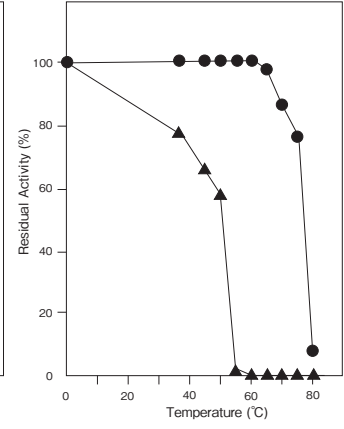


Fig.4 Thermal stability of GKZ



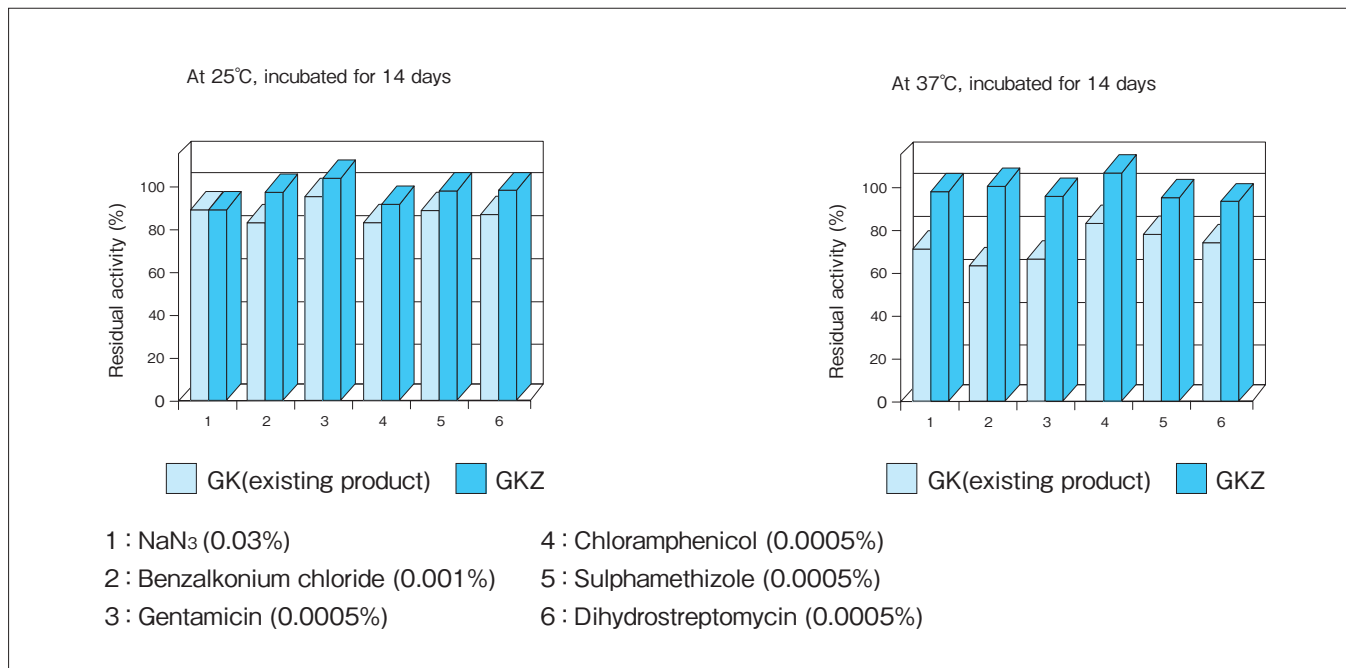
● : Phosphate buffer
○ : Tris-HCl buffer
▲ : Glycine-NaOH buffer

37°C, 60min
■ : 3,3-Dimethylglutarate-NaOH buffer
○ : Tris-HCl buffer
▲ : Glycine-NaOH buffer

pH 8.0
100 mM Tris-HCl buffer

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

Fig.5 Resistance of GKZ against antiseptics



1 : NaN₃ (0.03%)

2 : Benzalkonium chloride (0.001%)

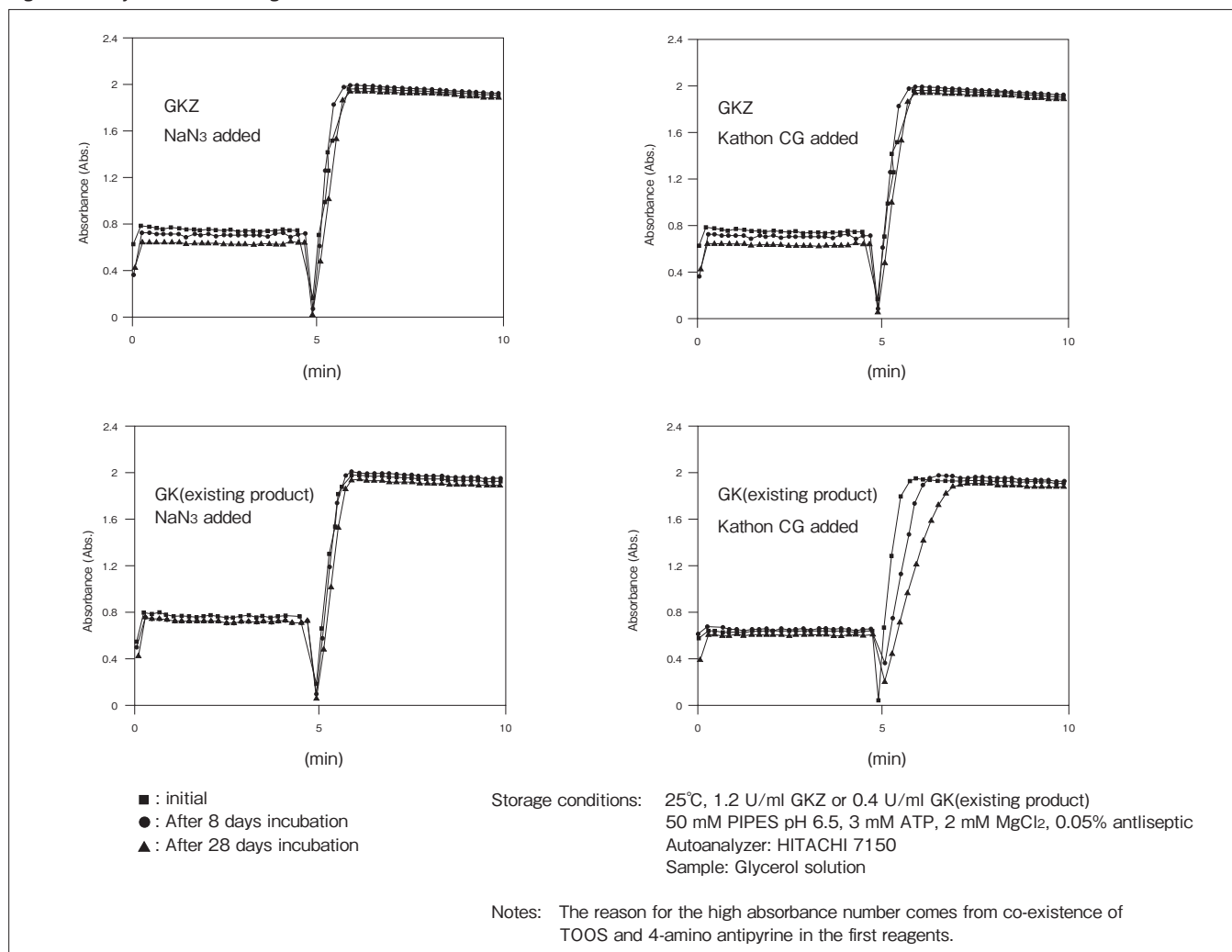
3 : Gentamicin (0.0005%)

4 : Chloramphenicol (0.0005%)

5 : Sulphamethizole (0.0005%)

6 : Dihydrostreptomycin (0.0005%)

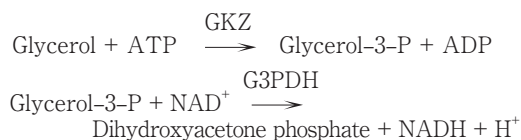
Fig.6 Reactivity of GKZ after long-term incubation



Assay

Principle

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



ATP: Adenosine triphosphate

NAD: Nicotinamide adenine dinucleotide

G3PDH: Glycerol-3-phosphate dehydrogenase

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

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 1.5mM MgCl₂
pH 9.8

Dissolve 12.5g hydrazine hydrate and 1.5g glycine with about 80ml distilled water. Add 1.5ml 0.1M MgCl₂ solution and adjust pH to 9.8 with 1N HCl at 25°C. Add distilled water to make a total of 100ml.

3. G3PDH solution

Use G3PDH (NH₄)₂SO₄ suspension (undiluted)

4. Enzyme dilution buffer

10 mM KH₂PO₄-NaOH buffer pH 7.0 containing
10 mM glycerol

5. Reagents

ATP: Kyowa Hakko Co., Ltd.

Hydrazine hydrate:

Tokyo Kasei Kogyo Co., Ltd. Purity 79% #H0204

NAD: NACALAI TESQUE, INC. #24334-84

G3PDH: Roche Diagnostics GmbH

(NH₄)₂SO₄ suspension #10127752001

■ 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 μ l of G3PDH, mix immediately.

After mixing, preincubate at 37°C.

2. After 5 min, add 30 μ l of enzyme solution and mix to start the reaction at 37°C.

※ In the case of a test blank, add 30 μ l 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

$$\Delta A/\text{min} = (A_s/\text{min} - A_b/\text{min}) \leq 0.050 \text{ Abs/min}$$

■ Calculation

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

3.3 : millimolar extinction coefficient of NADH at 366 nm
($\text{cm}^2/\mu\text{mole}$)

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°C 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)

ヒドラジン-水和物 (純度 79%) 12.5g とグリシン

1.5g および 0.1M 塩化マグネシウム溶液¹⁾

1.5ml を精製水 80ml に溶解した後、1N HCl で pH9.8 (25°C) 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。

又はヒドラジン-水和物 (純度 79%) 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°C) であればそのまま使用する。

3. G3PDH 溶液

ロシュ製の 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 \cdot 2Na \cdot 3H $_2$ O):

協和発酵製

ヒドラジン-水和物:

東京化成工業製 純度 79% #H0204

NAD (ニコチンアミドアデニンジヌクレオチド):

ナカライテスク製 #24334-84

G3PDH (グリセロ-3-リン酸脱水素酵素):

ロシュ製 硫酸懸濁液

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 μ l を分注して混和した後、直ちに 37°C で予備加温する。

2. 5分経過後、酵素試料液 30 μ l を正確に加えて混和し、直ちに 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 30 μ l 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。

$$\Delta A/\text{min} = (A_s/\text{min} - A_b/\text{min}) \leq 0.050 \text{ Abs/min}$$

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

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)