NIPRO ENZYMES

# GLYCEROKINASE (GlyK)

[EC 2.7.1.30]

from recombinant *E. coli*

Glycerol + ATP ↔ Glycerol-3-phosphate + ADP

## SPECIFICATION

| | | |
|---|---|---|
| State | : Lyophilized | |
| Specific activity | : more than 80 U/mg protein | |

## PROPERTIES

| | | |
|---|---|---|
| Subunit molecular weight | : ca. 54,700 | |
| Optimum pH | : 9.8 | (Fig. 1) |
| pH stability | : 5.5 - 9.0 | (Fig. 2) |
| Thermal stability | : No detectable decrease in activity up to 40 °C. | (Fig. 3) |
| Optimum temperature | : above 50 °C | (Fig. 4) |
| Michaelis constants | : (186 mM Glycine-Hydrazine-KOH buffer pH 9.8, at 30 °C) | |
| | Glycerol | 0.026 mM |
| | ATP | 0.025 mM |

## STORAGE

Stable at -20 °C for at least six months

## APPLICATION

The enzyme is useful for enzymatic determination of glycerol and triglyceride when coupled with glycerol-3-phosphate dehydrogenase

## ASSAY

### Principle

The change in absorbance is measured at 340 nm according to the following reactions.

$$\text{Glycerol + ATP} \xrightarrow{\text{GlyK}} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + NAD}^+ \xrightarrow{\text{G3PDH}} \text{Dihydroxyacetone phosphate + NADH + H}^+$$

### Unit Definition

One unit of activity is defined as the amount of GlyK that forms 1 μmol of NADH per minute at 30 °C.

### Solutions

Ⅰ Buffer solution ; 200 mM Glycine-Hydrazine-KOH, pH 9.8 (Dissolve 1.5 g glycine and 5 mL hydrazine hydrate in 80 mlmL distilled water. After adjusting pH to 9.8 with 1 M KOH, fill up to 100 mL with distilled water.)

Ⅱ $MgCl_2$ solution ; 100 mM (2.03 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)

Ⅲ ATP solution ; 100 mM (0.605 g ATP disodium salt$\cdot 3H_2O$/(8.2 mL distilled water + 1.8 mL 1 M NaOH))

Ⅳ $NAD^+$ solution; 100 mM (0.663 g $NAD^+$ free acid/10 mL distilled water)

Ⅴ Glycerol-3-phosphate dehydrogenase ; 1700 U/mL (from rabbit muscle, Roche Diagnostics)

Ⅵ Glycerol solution ; 330 mM (3.04 g Glycerol/100 mL distilled water)

### Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute the enzyme solution to 0.1 to 1.0 U/mL with 50 mM Tris-HCl buffer pH 9.0 containing 0.1 % bovine serum albumin.

### Procedure

1. Prepare the following reaction mixture and pipette 2.70 mL of reaction mixture into a cuvette.

| | | | |
|---|---|---|---|
| Solution Ⅰ | 27.90 mL | SolutionⅣ | 0.15 mL |
| Solution Ⅱ | 0.57 mL | Solution Ⅴ | 0.30 mL |
| SolutionⅢ | 0.39 mL | SolutionⅥ | 0.30 mL |
| Distilled water | 0.39 mL | | |

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.015 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute ($\Delta Abs_{340}$) in the linear portion of curve.

### Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{340}) \text{ X } (2.70 + 0.015)}{6.22 \text{ X } 0.015} \text{ X d.f.}$$

$$\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)*}}$$

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH ($cm^2$/μmol)
*Protein concentration ; determined by the absorbance at 280 nm (Abs280), where 1 Abs280 = 1 mg/mL

## REFERENCE

1. Mike J. Comer, Chris J. Bruton, and Tony Atkinson ; *J. App. Biochem.* **1**, 259-270 (1979)

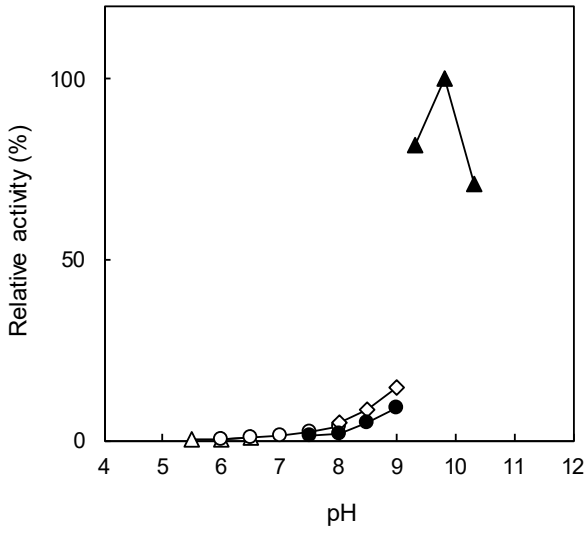


Fig. 1 pH profile

△ MES, ○ phosphate,
● Tris-HCl, ◇ Bicine,
▲ Glycine-Hydrazine

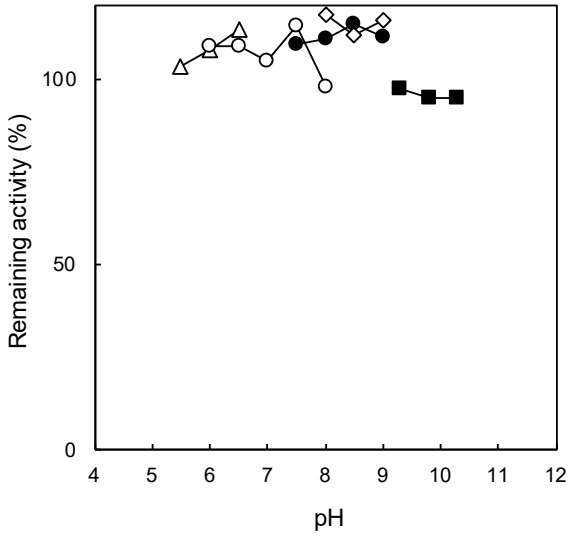


Fig. 2 pH stability

treated for 24 hr at 4 ℃ in the following buffer solution (0.1 M);
△ MES, ○ phosphate,
● Tris-HCl, ■ Glycine-Hydrazine, ◇ Bicine

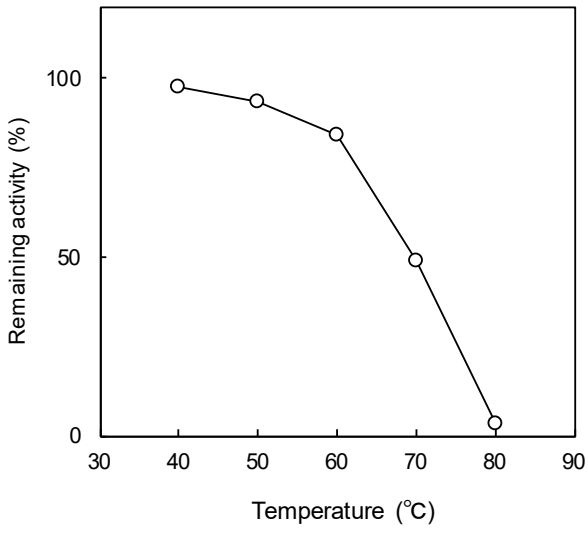


Fig. 3 Thermal stability

treated for 15 min in 100 mM Bicine buffer, pH 8.5

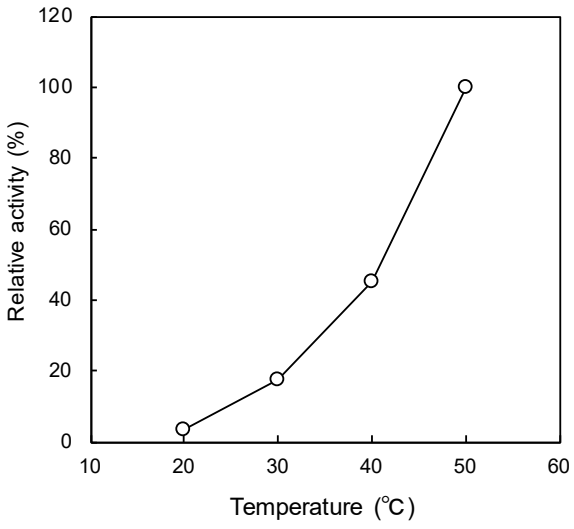


Fig. 4 Thermal activity