



## 重组甘油激酶 (GK)

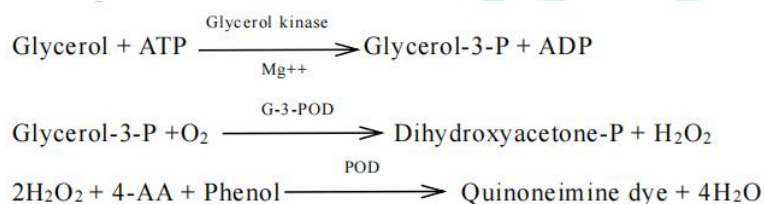
Glycerol Kinase (GK, EC 2.7.1.30) is a key member of the kinase family, catalyzing the initial reaction in glycerol metabolism. In the presence of ATP, this enzyme can specifically catalyze the phosphorylation of glycerol, generating glycerol-3-phosphate (G-3-P) and ADP.

### Application

In clinical analysis, this enzyme can be used for the enzymatic determination of glycerol and triglycerides by coupling with glycerol-3-phosphate oxidase, or with pyruvate kinase and lactate dehydrogenase, or with lipoprotein lipase.

### Assay procedure

#### Principle



The appearance of quinoneimine dye is measured at 500nm by spectrophotometry.

#### Unit definition

One unit causes the formation of one micromole of hydrogen peroxide(half a micromole of quinoneimine dye)per minute under the conditions described below.

#### Reagents

A. Glycerol solution:0.3M(Should be prepared fresh)

B. 4-AA solution:0.1%(100mg of 4-aminoantipyrine/100ml of  $\text{H}_2\text{O}_2$ )



C. Phenol solution: 0.1% (100mg of phenol/100ml of  $\text{H}_2\text{O}_2$ )

D. Peroxidase solution: 20mg Peroxidase (110 purpurogallin unit/mg)/100ml of  $\text{H}_2\text{O}_2$

E. G-3POD Solution: 20U/ml (dissolve in 200Mm HEPES buffer, pH 7.9)

F. Buffer solution: 200Mm HEPES, pH 7.9 contg. 20Mm  $\text{MgCl}_2$  and 40mM ATP  
(Should be prepared fresh)

G. Enzyme diluent: 20mM K-phosphate buffer, pH 7.5

## Procedure

1. Prepare the following working solution in a brownish bottle and store on ice.

|      |                     |     |
|------|---------------------|-----|
| 10ml | 4-AA solution       | (B) |
| 20ml | Phenol solution     | (C) |
| 20ml | Peroxidase solution | (D) |
| 40ml | G-3POD solution     | (E) |
| 10ml | Buffer solution     | (F) |

Concentration in assay mixture



|              |              |
|--------------|--------------|
| HEPES buffer | 95.2mM       |
| Glycerol     | 4.76mM       |
| ATP          | 3.81mM       |
| MgCl 2       | 1.90mM       |
| 4-AA         | 0.469mM      |
| Phenol       | 2.02mM       |
| G-3-POD      | ca.7.6 U /ml |
| POD          | ca.5.2 U /ml |

2. Pipette 3.0ml of working solution into a cuvette (d=1.0cm). (d=1.0 cm)
3. Add 0.1ml of the enzyme solution\* mix by gentle inversion and equilibrate at 37°C for about 5 minutes.
4. Add 0.05ml of glycerol solution(A)and mix by gentle inversion.
5. Record the optical density at 500nm against water for 3 to 4 minutes in a spectrophotometer thermostated at 37°C,and calculate the  $\Delta OD$  per minute from the initial portion of the curve( $\Delta OD$  test).

At the same time,measure the blank rate( $\Delta OD$  blank)by using the same method as the test except that enzyme diluent (G) is added instead of the enzyme solution.

\* Dissolve the enzyme preparation in ice-cold enzyme diluent(G)and dilute to 0.2~0.4U/ml with the same buffer,immediately before assay.

## Calculation



Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{\Delta \text{OD/min}(\text{OD}_{\text{test}} - \text{OD}_{\text{blank}}) \times V_t \times \text{df}}{13.3 \times \frac{1}{2} \times 1.0 \times V_s} = \Delta \text{OD/min} \times 4.74 \times \text{df}$$

$$\text{Weight activity (U/mg)} = (\text{U/ml}) \times 1/\text{C}$$

V<sub>t</sub>: Total volume (3.15ml)

V<sub>s</sub>: Sample volume (0.1ml)

13.3: Millimolar extinction coefficient of quinoneimine dye under the assay conditions (cm<sup>2</sup>/μmol)

1.0: Light path length (cm)

1/2: Factor based on the fact that one mole of **H<sub>2</sub>O<sub>2</sub>** produces a half of quinoneimine dye.

df: Dilution factor

C: Enzyme concentration in dissolution (C mg/ml)