ENZYMES

Glycerol Kinase

ORIGIN *Cellulomonas sp.*

CAT# GLKI-70-6495
EC# 2.7.1.30

SPECIFICATIONS

Appearance	White/off-white free flowing powder
Activity	>30 U/mg powder at 25°C

ASSAY PRINCIPLE

Glycerol Kinase (GK) catalyses the following reaction:

$$\text{Glycerol} + \text{ATP} \xrightarrow[Mg^{++}]{GK} \text{Glycerol-3-P} + \text{ADP}$$

$$\text{Glycerol-3-P} + NAD^{+} \xrightarrow{\text{Glycerol-3-PDH}} \text{Dihydroxyacetone-P} + \text{NADH} + H^{+}$$

The appearance of NADH is measured spectrophotometrically at 340nm.

UNIT DEFINITION

One unit of activity is defined as the amount of enzyme that will catalyse the phosphorylation of 1.0 micromole of glycerol per minute at 25°C under standard assay method conditions.

APPLICATION

Useful for the measurement of Triglyceride.

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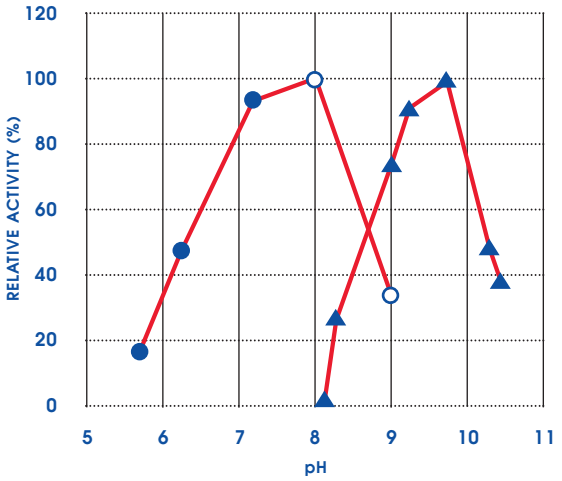
CHARACTERISTICS

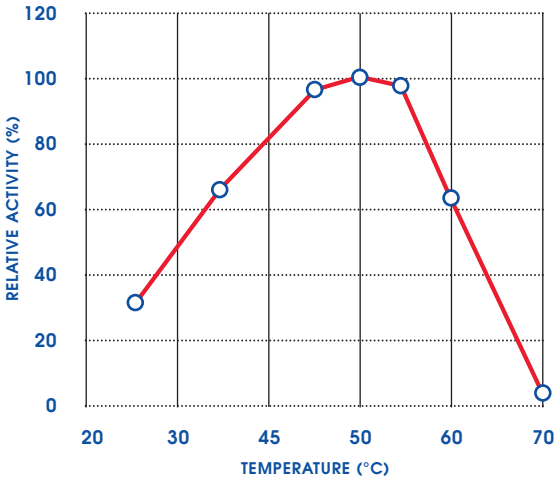
Molecular Weight:	128kDa (gel filtration)
Isoelectric Point:	4.2
K_m values:	**Glycerol** 4.4 x 10^{-5} M **ATP** 4.3 x 10^{-4} M
Optimum pH (Fig. 1):	**G-3-PDH system** 9.8 **G-3-P oxidase system** 7.8
Optimum Temperature (Fig. 2):	50°C
pH Stability (Fig. 3):	5.5 to 10.0 (25°C for 20 hours)
Thermal Stability (Fig. 4):	Below 40°C (pH 7.5 for 15 minutes)

FIGURE 1: OPTIMUM pH



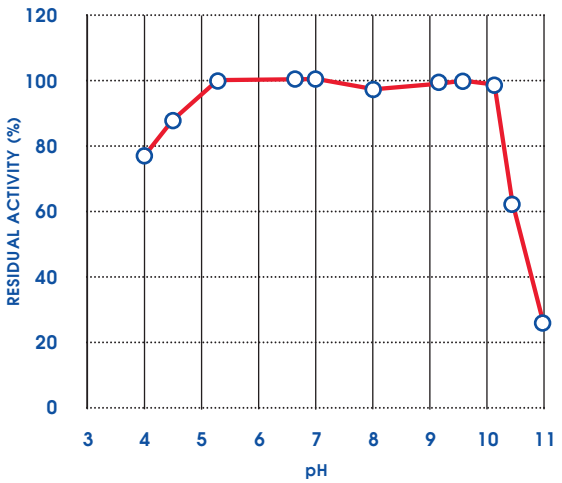
● **25°C, G-3-P OXIDASE SYSTEM,** 50mM (2-N-Morpholino) ethanesulfonic acid-NaOH buffer
○ **25°C, G-3-P OXIDASE SYSTEM,** 50mM Tris-HCl buffer
▲ **25°C, G-3-PDH SYSTEM,** 0.18M glycine-hydrazine buffer

FIGURE 2: OPTIMUM TEMPERATURE



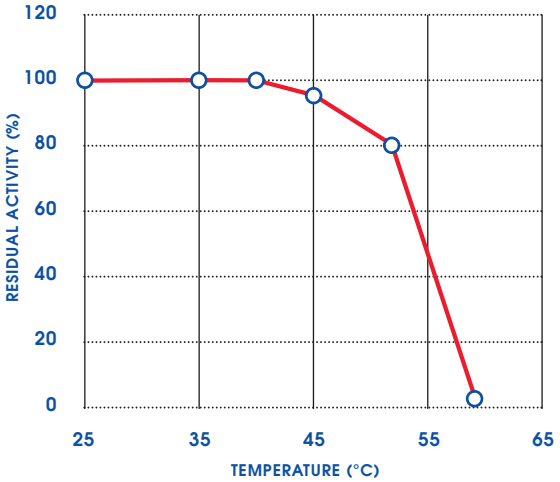
0.18M glycine-hydrazine buffer, pH 9.8

FIGURE 3: pH STABILITY



25°C: 20hr. treatment with 50mM buffer solution: pH 4.0-6.0, acetate; pH 6.0-9.0, K-phosphate; pH 9.0-11.0, K_2CO_3-$NaHCO_3$

FIGURE 4: THERMAL STABILITY



15min. treatment with 50mM potassium phosphate buffer, pH 7.5

TABLE 1: THE SUBSTRATE SPECIFICITY OF GLYCEROL KINASE (PYRUVATE KINASE-LACTATE DEHYDROGENASE SYSTEM, PH 7.5)

SUBSTRATE (6mM)	RELATIVE ACTIVITY (%)	SUBSTRATE (6mM)	RELATIVE ACTIVITY (%)
Glycerol	100	2, 3-Butanediol	—
Glycerol-α-monochlorohydrin	0.09	D-Mannitol	—
Ethylene glycol	—	D-Sorbitol	—
1, 2-Propanediol	—	D-Glucose	—
1, 3-Propanediol	0.07	Ribitol	—
1, 3-Butanediol	—	Methanol	—
1, 4-Butanediol	—	Ethanol	—

(—) Not detected

TABLE 2: THE EFFECT OF VARIOUS CHEMICALS ON GLYCEROL KINASE

CHEMICAL	CONCENTRATION	RELATIVE ACTIVITY (%)	CHEMICAL	CONCENTRATION	RELATIVE ACTIVITY (%)
Triton X-100	0.1%	99	$AgNO_3$	2 mM	0
Sodium Cholate	0.1%	97	$HgCl_2$	2 mM	0
$MgCl_2$	2 mM	100	p-Chloromer-curibenzoate	2 mM	22
$CaCl_2$	2 mM	100	Monoiodoacetate	2 mM	96
$Ba(OCH_3CO)_2$	2 mM	100	Sodium Fluorite	2 mM	97
$FeCl_3$	2 mM	75	Sodium Azide	20 mM	97
$CoCl_2$	2 mM	100	EDTA	5 mM	101
$MnCl_2$	2 mM	100	o-Phenanthroline	2 mM	96
$Zn(OCH_3CO)_2$	2 mM	99	α, α-Dipyridyl	2 mM	92
$NiCl_2$	2 mM	98	Borate	50 mM	100
$CuSO_4$	2 mM	100			
$Pb(OCH_3CO)_2$	2 mM	88			

THE AMERICAS
Sekisui Diagnostics, LLC
4 Hartwell Place
Lexington, MA 02421
Phone: 800 332 1042
Fax: 800 762 6311

INTERNATIONAL
Sekisui Diagnostics (UK) Limited
Liphook Way, Allington
Maidstone, Kent, ME16 0LQ, UK
Phone: +44 1622 607800
Fax: +44 1622 607801

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engage@sekisuienzymes.com
www.sekisuienzymes.com