



Glycerol Kinase
Catalogue No. GLKI-70-6495, 70-6495-01

Origin: *Cellulomonas sp.*

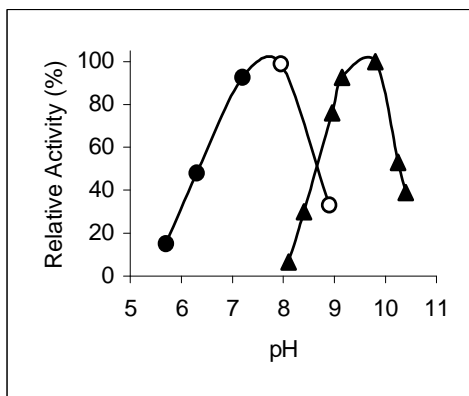
Specifications:

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

Characteristics:

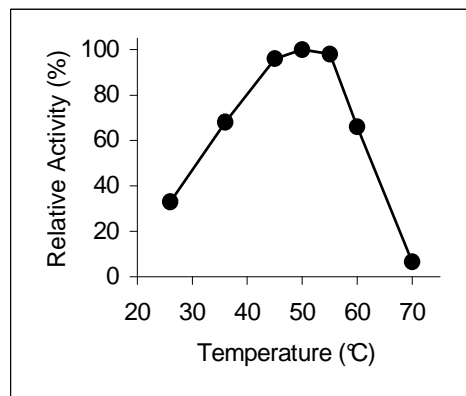
Molecular Weight:	128kDa (gel filtration)	
Isoelectric point:	4.2	
K _m values:	Glycerol 4.4 x 10 ⁻⁵ M	
	ATP 4.3 x 10 ⁻⁴ M	
Optimum pH:	9.8 (G-3-PD system)	See Fig. 1
	7.8 (G-3-P oxidase system)	
Optimum temp.:	50°C	See Fig. 2
pH stability:	5.5-10.0 (25°C, 20 hr.)	See Fig. 3
Thermal stability:	Below 40°C (pH 7.5, 15 min.)	See Fig. 4
Substrate specificity:		See table 1
Effect of chemicals:		See table 2
Lyophilised stability:	1 year at -20°C	

Fig. 1 pH Optimum



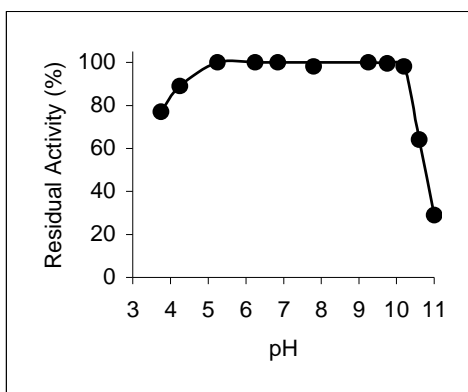
▲ : 25°C, G-3-PD system, 0.18M glycine-hydrazine buffer
○ : 25°C, G-3-P oxidase system, 50mM Tris-HCl buffer
● : 25°C, G-3-P oxidase system, 50mM (2-N-Morpholino) ethanesulfonic acid-NaOH buffer

Fig. 2 Temperature Optimum

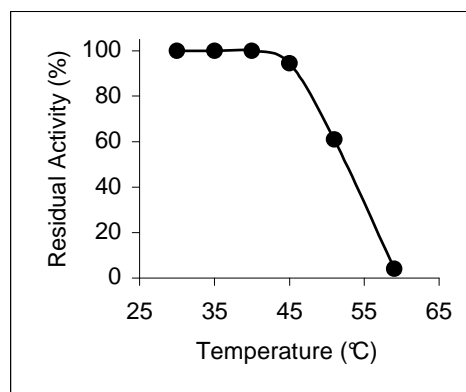


0.18M glycine-hydrazine buffer, pH 9.8

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Fig. 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, K₂CO₃-NaHCO₃

Fig. 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 (%)
Glycerol	100
Glycerol - α - monochlorohydrin	0.09
Ethylene glycol	-
1, 2 - Propanediol	-
1, 3 - Propanediol	0.07
1, 3 - Butanediol	-
1, 4 - Butanediol	-
2, 3 - Butanediol	-
D - Mannitol	-
D - Sorbitol	-
D - Glucose	-
Ribitol	-
Methanol	-
Ethanol	-

(-) Not detected



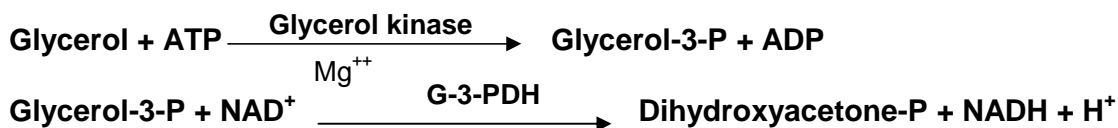
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Table 2. The effect of various chemicals on Glycerol Kinase:

Chemical	Concentration	Relative Activity (%)
Triton X-100	0.1%	99
Sodium Cholate	0.1%	97
MgCl ₂	2 mM	100
CaCl ₂	2 mM	100
Ba(OCH ₃ CO) ₂	2 mM	100
FeCl ₃	2 mM	75
CoCl ₂	2 mM	100
MnCl ₂	2 mM	100
Zn(OCH ₃ CO) ₂	2 mM	99
NiCl ₂	2 mM	98
CuSO ₄	2 mM	100
Pb(OCH ₃ CO) ₂	2 mM	88
AgNO ₃	2 mM	0
HgCl ₂	2 mM	0
p-Chloromercuribenzoate	2 mM	22
Monoiodoacetate	2 mM	96
Sodium Fluoride	2 mM	97
Sodium Azide	20 mM	97
EDTA	5 mM	101
o - Phenanthroline	2 mM	96
α, α - Dipyridyl	2 mM	92
Borate	50 mM	100

Assay Principle:

Glycerol Kinase catalyses the following reaction:



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.
(See *Analytical Method* for full details)

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