rGlDH(NADP)

recombinant Glutamate dehydrogenase (NADP+) EC 1.4.1.4

from Bacteria

Reaction Equation

L-Glutamate + H_2O + $NADP^+$

$\rightleftarrows$ α-Ketoglutarate + NH_3 + NADPH + H^+

Specification

Specific Activity

U/mg protein	> 60 units

(for reduction of α-Ketoglutarate to L-Glutamate)

Contaminants

Glucose 6-phosphate dehydrogenase	< 0.02%
Phosphogluconate dehydrogenase	< 0.1%
Glutamate dehydrogenase (NAD^+)	< 0.03%
Glutathione reductase	< 0.02%
NADPH oxidase	< 0.003%

Properties

pH stability	: pH 5.0 - 10.5 (25°C, 1 week)
Thermal stability	: ≦ 70°C (pH 7.5, 10 min)
Optimum pH	: 7.5 - 8.0
Optimum temp.	: ≧ 70°C
Km value	: 2.5×10^{-2} mol/L (L-Glutamate)
	6.4×10^{-5} mol/L ($NADP^+$)
	4.1×10^{-4} mol/L (α-Ketoglutarate)
	2.4×10^{-5} mol/L (NADPH)
	4.7×10^{-5} mol/L (Ammonium acetate)
Molecular weight	: 46 kDa (SDS-PAGE)

Assay Procedure

I Spectrophotometric Method

Wavelength : 340 nm, Light path length : 1 cm
Final volume : 3.02 mL, Temperature : 25°C

Pipette the following reagents into a cuvette

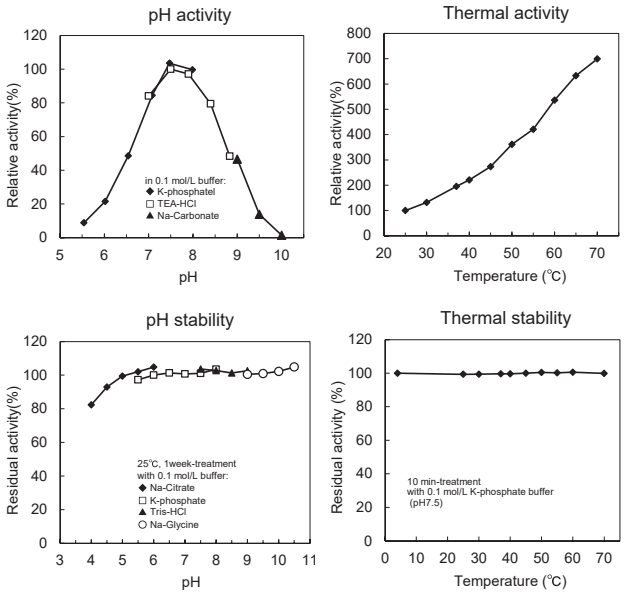
2.50 mL	Triethanolamine-HCl buffer (0.1 mol/L, pH 7.6)
0.15 mL	α-Ketoglutarate (0.1 mol/L)
0.05 mL	NADPH (12 mmol/L)
0.30 mL	Ammonium acetate (2 mol/L)
0.02 mL	rGlDH(NADP) (approx. 3 U/mL)

II Calculation

$$\frac{\Delta\ A/min \cdot V \cdot D}{6.2 \cdot d \cdot v} = U/mL$$

Δ A/min = The change in absorbance at 340 nm/minute
V = Total volume of reaction mixture (3.02 mL)
D = Enzyme dilution factor
6.2 = mmol/L extinction coefficient of NADPH
($L \cdot mmol^{-1} \cdot cm^{-1}$)
d = Light path length (1 cm)
v = Volume of enzyme sample (0.02 mL)

Reference Data



Preparation and Storage

Solution
Store at 1 - 10°C

Cat. No./Package

Cat. No.	Package
46754904	Bulk

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