rHK(Y)

recombinant Hexokinase EC 2.7.1.1

from Yeast

Reaction Equation

ATP + D-Hexose → ADP + D-Hexose 6-phosphate

Specification

Specific Activity

U/mg protein	> 180 units

Contaminants

Phosphoglucose isomerase	< 0.003%
Glutathione reductase	< 0.005%
Myokinase	< 0.001%
Phosphogluconate dehydrogenase	< 0.001%
Phosphoglucomutase	< 0.001%
Glucose 6-phosphate dehydrogenase	< 0.005%
Creatine kinase	< 0.005%
ATPase	< 0.003%

Properties

pH stability : pH 5.0 - 8.0 (25 °C, 1 week)
Thermal stability : ≦ 40 °C (pH 7.5, 10 min)
Optimum pH : 7.5 - 9.0
Optimum temp. : 50 °C
Km value : 1.8 × 10^{-4} mol/L (ATP)
2.9 × 10^{-4} mol/L (Glucose)
Molecular weight : 54 kDa (SDS-PAGE)

Assay Procedure

I Spectrophotometric Method

Wavelength : 340 nm, Light path length : 1 cm
Final volume : 3.03 mL, Temperature : 25 °C

Pipette the following reagents into a cuvette

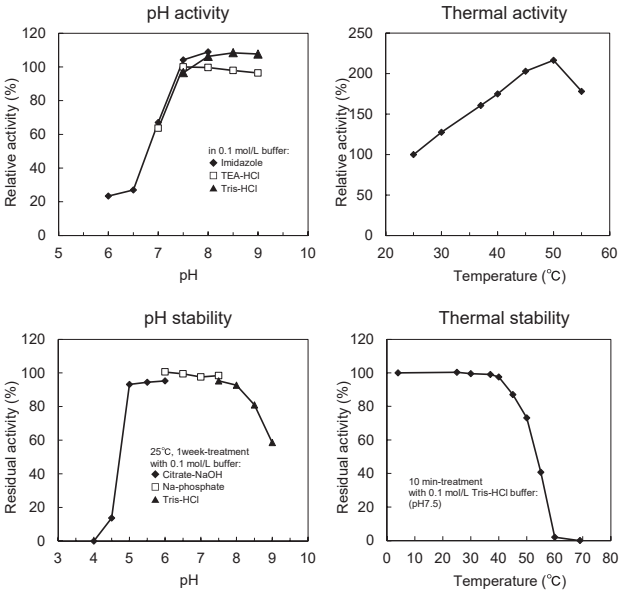
2.40 mL	Triethanolamine-HCl buffer(0.1 mol/L, pH 7.5) containing Glucose (50 mg/mL)
0.30 mL	$MgCl_2$ (0.1 mmol/L)
0.15 mL	ATP (10 mmol/L)
0.15 mL	$NADP^+$ (10 mmol/L)
0.01 mL	G6PDH (500 U/mL)
0.02 mL	rHK (Y) (approx. 3 U/mL)

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 (3.03 mL)
D = Enzyme dilution factor
6.2 = mmol/L extinction coefficient of NADPH
(L·$mmol^{-1}$·cm^{-1})
d = Light path length (1 cm)
v = Volume of enzyme sample (0.02 mL)

Reference Data



Preparation and Storage

Lyophilized powder
Store below -20 °C

Cat. No./Package

Cat. No.	Package
46763900	Bulk

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