

Product Information

Hexokinase

from *Saccharomyces cerevisiae*

Catalog Number **H4502**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

CAS RN 9001-51-8

EC 2.7.1.1

Synonyms: ATP:D-hexose 6-phosphotransferase

Product Description

Glycolysis is the process in almost all living organisms by which D-glucose is metabolized to generate energy and metabolic intermediates. In the first step of glycolysis, hexokinase (HK) phosphorylates the C6 position of D-glucose in the presence of ATP:



Yeast hexokinase has three isozymes, designated P-I, P-II, and glucokinase (Glk1), each with distinctive properties.¹ Yeast hexokinase P-II has both catalytic and regulatory functions.² Hexokinase is a dimeric protein with two 55 kDa monomers, for an overall molecular mass of 110 kDa.^{3,4}

Hexokinase is used for the determination of D-glucose, D-fructose,⁵ and D-sorbitol in food or other biological materials.⁵ Hexokinase can also be used in the assay of glycosides that are convertible to glucose or fructose.

pI:⁶

P-I: 5.25

P-II: 4.93

Several other hexoses can serve as substrates for hexokinase, with the following relative reaction rates:⁷

Glucose	1.0
2-deoxy-2-fluoro-D-glucose	0.5
Mannosamine	0.2
5-thioglucoase	0.01
3-deoxy-3-aminoglucoase	0.003

Other potential substrates include 1-thio-D-glucose and 1,5-anhydro-D-glucitol.^{1,7}

K_M (mM):^{1,7}

D-glucose	0.12 (P-I and P-II)
D-fructose	0.33 (P-I and P-II)
D-mannose	0.04 (Glk1)
D-mannosamine	5
5-thio-D-glucose	4

Extinction coefficient:

P-I: $E_{280}^{1\%} = 8.85$

P-II: $E_{280}^{1\%} = 9.47$

pH Optimum:⁸ 7.5–9.0

Activators of hexokinase include Mg^{2+} ($K_M = 2.6\text{ mM}$) and catecholamine-related compounds.

Inhibitors^{1,7}

Inhibitors ^{1,7}	K_i (mM)
D-glucosamine	1.5
D-mannose	0.06
D-xylose	25 (isozyme P-I) 80 (isozyme P-II)
6-deoxy-D-glucose	50
N-acetylmannosamine	50

Other inhibitors include sorbose-1-phosphate, polyphosphates, 6-deoxy-6-fluoroglucoase, 2-C-hydroxymethylglucose, lyxose, and thiol-reactive compounds.⁸⁻¹⁰

This product is purified from *Saccharomyces cerevisiae* (baker's yeast). It is a mixture of isozymes, and is supplied as a sulfate-free lyophilized powder.

Protein: $\geq 70\%$, balance primarily sodium citrate

Specific activity: ≥ 130 units/mg protein (Biuret)

Unit Definition: One unit will phosphorylate 1.0 μmole of D-glucose per minute at pH 7.6 at $25\text{ }^{\circ}\text{C}$.

Sigma assays hexokinase spectrophotometrically in a 2.57 mL reaction mixture containing 39 mM triethanolamine, 216 mM D-glucose, 0.74 mM ATP, 7.8 mM MgCl_2 , 1.1 mM $\beta\text{-NADP}$, 2.5 units glucose-6-phosphate dehydrogenase, and 0.025–0.05 units hexokinase.

Contaminants:ATPase ($\leq 0.01\%$)Myokinase ($\leq 0.01\%$)Glucose-6-phosphate dehydrogenase ($\leq 0.01\%$)6-Phosphogluconic dehydrogenase ($\leq 0.01\%$)Phosphoglucose isomerase ($\leq 0.01\%$)**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Hexokinase is soluble in cold water (0.5-1.0 unit/mL) or citrate buffer, pH 7.0.

Storage/Stability

The product should be stored at $-20\text{ }^{\circ}\text{C}$.

Solutions of hexokinase in water or citrate buffer have retained activity during repeated freezing and thawing for a period of 30 days. In general, stock solutions of hexokinase should be stored at neutral pH.¹¹

References

1. Fernández, R., *et al.*, *J. Gen. Microbiol.*, **131(10)**, 2705-2709 (1985).
2. Entian, K.-D., and Fröhlich, K.-U., *J. Bacteriol.*, **158(1)**, 29-35 (1984).
3. Easterby, J.S., and Rosemeyer, M.A., *FEBS Lett.*, **4(2)**, 84-86 (1969).
4. Jacob, L., *et al.*, *J. Chromatogr.*, **587(1)**, 85-92 (1991).
5. Bellaloui, N., *et al.*, *Front. Plant Sci.*, **6**, 31 (2015).
6. Schmidt, J.J., and Colowick, S.P., *Arch. Biochem. Biophys.*, **158(2)**, 471-477 (1973).
7. Machado de Domenech, E. E., and Sols, A., *FEBS Lett.*, **119(1)**, 174-176 (1980).
8. Sols, A., *et al.*, *Biochem. Biophys. Acta*, **30(1)**, 92-101 (1958).
9. Harrison, W.H., *et al.*, *Lancet*, **2(2771)**, 277 (1972).
10. Mulcahy, P., *et al.*, *Anal. Biochem.*, **309(2)**, 279-292 (2002).
11. Souza, M.A., *et al.*, *Appl. Biochem. Biotech.*, **98-100(1-9)**, 265-272 (2002).

GCY,MB,JWM,MAM 10/17-1