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Product Information

Hexokinase
from *Saccharomyces cerevisiae*

Catalog Number **H6380**
Storage Temperature 2–8 °C

CAS RN 9001-51-8
EC 2.7.1.1
Synonyms: ATP:D-hexose 6-phosphotransferase

Product Description
Glycolysis is the process occurring in almost all living organisms by which they metabolize D-glucose to generate energy and metabolic intermediates. In the first step of glycolysis, hexokinase phosphorylates the C6 position of D-glucose in the presence of ATP by the following reaction:

$$\text{ATP} + \text{D-glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{D-glucose 6-phosphate}$$

Yeast hexokinase has three isozymes, designated P-I, P-II, and glucokinase (Glk1). Each has distinctive properties.[1] Yeast hexokinase P-II has both a catalytic and a regulatory function.[2]

pI:[3]

P-I	5.25
P-II	4.93

Several other hexoses can serve as substrates for hexokinase (relative reaction rates):[4]

glucose	1.0
2-deoxy-2-fluoro-D-glucose	0.5
mannosamine	0.2
5-thioglucose	0.01
3-deoxy-3-aminoglucose	0.003

Also:[1,4] 1,5-anhydro-D-glucitol, 1-thio-D-glucose

K_M (mM):[1,4]

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

Molecular Mass:[5] 110 kDa (dimer)

Hexokinase is a dimeric protein with two equal 55 kDa monomers.

Extinction coefficient:

P-I	$E_{280}^{1\%} = 8.85$
P-II	$E_{280}^{1\%} = 9.47$

pH Optimum:[6] 7.5–9.0

Activators: Mg^{2+} (K_M = 2.6 mM), catecholamine-related compounds[7]

Inhibitors[1,4]

	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-acetylmannoseamine	50

Also sorbose-1-phosphate, polyphosphates, 6-deoxy-6-fluoroglucose, 2-C-hydroxy-methylglucose, lyxose, and thiol reactive compounds[6]

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

This product (Catalog Number H6380) is purified from overproducing *Saccharomyces cerevisiae* (baker's yeast). It is supplied as a lyophilized powder and is predominantly the P-II isoform.

Protein: ~15%

Specific activity: ≥350 units/mg protein (biuret)

Unit Definition: One unit will phosphorylate 1.0 µmole of D-glucose per min at pH 7.6 at 25 °C.

Hexokinase is assayed 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 β–NADP, 2.5 units glucose-6-phosphate dehydrogenase, and 0.025–0.05 unit hexokinase.

Contaminants:
phosphoglucose isomerase (≤0.002%)
alcohol dehydrogenase (≤0.001%)
creatine phosphokinase (≤0.001%)
myokinase (≤0.001%)
6-phosphogluconic dehydrogenase (≤0.001%)
glutathione reductase (≤0.005%)
L-glutamic dehydrogenase (≤0.05%)
glucose-6-phosphate dehydrogenase (≤0.005%)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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
Store the product at 2–8 °C and it remains active for at least 2 years when stored properly.

Solutions in water or citrate buffer have remained active during repeated freezing and thawing for a period of 30 days.

References

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6. Sols, A., *et al.*, Substrate specificity and some other properties of baker's yeast hexokinase. Biochem. Biophys. Acta, **30**, 92-101 (1958).
7. Harrison, W.H., *et al.*, Aluminium inhibition of hexokinase. Lancet, **2**, 277 (1972).
8. Mulcahy, P., *et al.*, Application of kinetic-based biospecific affinity chromatographic systems to ATP-dependent enzymes: Studies with yeast. Anal. Biochem., **309,** 279-92, (2002).

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