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ProductInformation

Hexokinase
from *Saccharomyces cerevisiae*

Catalog Number **H4502**
Storage Temperature –20 °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 Weight:[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 H4502) is purified from *Saccharomyces cerevisiae* (baker's yeast). It is supplied as a sulfate-free lyophilized powder. It is a mixture of isozymes.

Protein: ≥85%, balance primarily sodium citrate.

Specific activity: ≥130 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:
ATPase (≤0.01%)
Myokinase (≤0.01%)
glucose-6-phosphate dehydrogenase (≤0.01%)
6-phosphogluconic dehydrogenase (≤0.01%)
phosphoglucose isomerase (≤0.01%)

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 –20 °C and it is stable for at least 2 years when stored properly.

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

References

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3. Schmidt, J.J., and Colowick, S.P., Identification of a peptide sequence involved in association of subunits of yeast hexokinases. Arch. Biochem. Biophys., **158,** 471-77 (1973).
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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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