For life science research only.
Not for use in diagnostic procedures.

Hexokinase (HK)
from yeast overproducer

Version: 12
Content Version: November 2021

Suspension in ammonium sulphate.

Cat. No. 11 426 362 001	1,500 U 1 ml

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Hexokinase	Suspension in 3.2 M ammonium sulphate.	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Hexokinase	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

For measuring Hexokinase activity

- NADP*
- Glucose
- Triethanolamine buffer
- $MgCl_2$
- ATP*
- Glucose-6-Phosphate Dehydrogenase*

1.4. Application

Use Hexokinase for the determination of D-glucose, D-fructose, and D-sorbitol in food or biological research samples. The enzyme is also used for the assay of other saccharides which are convertible to glucose or fructose, and is therefore useful in the assay of many glycosides.

2. How to Use this Product

2.1. Before you Begin

General Considerations

If Hexokinase is used in combination with glucose-6-phosphate dehydrogenase (G6P-DH)* (assays glucose-6-phosphate formed by Hexokinase), samples should not be of high phosphate concentrations as G6P-DH is competitively inhibited by phosphate.

Although phosphate may be used to buffer assay systems using Hexokinase and G6P-DH, a more suitable buffer for these assays at pH 7.6 to 8.0 is triethanolamine hydrochloride.

pH optimum

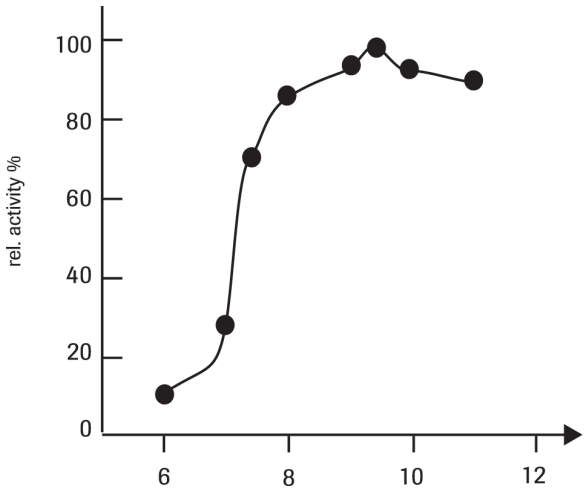

Fig. 1: 0.1 M Triethannolamine buffer, +25°C, assay as described.

pH stability

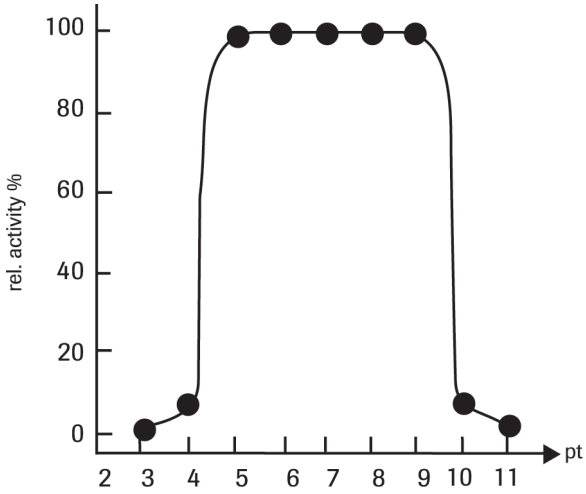

Fig. 2: 3 hours at +25°C, 800 U/ml, pH 3 to 5: citrate/phosphate buffer, pH 6 to 8: potassium phosphate buffer, pH 9 to 11: glycine buffer.

2.2. Parameters

Activator

Requires Mg^{2+} ions (K_m = 2.6 mM) for activity. Catecholamines also increase activity.

Contaminants

Contaminant	Activity [%]
Creatine kinase	<0.001% (creatine as substrate; pH 9.0, +25°C)
Glucose-6-phosphate dehydrogenase	<0.005% (glucose-6-phosphate as substrate; pH 7.6, +25°C)
Myokinase	<0.001% (ATP and AMP as substrates; pH 7.6, +25°C)
6-phosphogluconate dehydrogenase	<0.001% (gluconate-6-phosphate as substrate; pH 7.6, +25°C)
Phosphoglucoseisomerase	<0.002% (fructose-6-phosphate as substrate; pH 7.6, +25°C)
Glutamate dehydrogenase	<0.05% (α-ketoglutarate as substrate and NADPH as coenzyme; pH 7.9, +25°C)
ATPase	<0.05% (ATP as substrate; pH 7.6, +25°C)
Glutathione reductase	<0.005% ($glutathione_{ox}$ as substrate; pH 8.0, +95°C)
Alcohol dehydrogenase	<0.001% (ethanol as substrate; pH 9.0, +25°C)
Glucose	≤30 µg/ml (enzymatic)

Inhibition

Inhibitors

- DTA
- Thiol blocking agents, such as Hg^{2+} and 4-chloromercuribenzoate
- Polyphosphates
- Lyxose
- Sorbose-1-phosphate
- 6-deoxy-6-fluoroglucose
- Glucose-6-phosphate

K_i = 9.1 mM, pH 8.0, +25°C.

Molecular Weight

Hexokinase consists of 486 amino acids and has a molecular weight of 57 kDa (SDS-PAGE) in citrate/phosphate buffer. It may form dimers under other buffer conditions.

Specific Activity

>450 U/mg protein (D-glucose and ATP* as substrates, +25°C, pH 7.6).

Specificity

Hexokinase catalyzes the phosphorylation of hexoses with different rates (pH 7.5, +30°C):

Substrate	K_m value [mM]	Relative rate
D-glucose	0.1	1.0
D-fructose	0.7	1.8
D-mannose	0.05	0.8
D-glucosamine	1.5	0.7
2-deoxy-D-glucose	0.3	1.0

Sugars not phosphorylated

- L-arabinose
- D-xylose
- D-lyxose
- L-rhamnose
- D-galactose
- Sucrose
- Lactose
- Maltose
- Trehalose
- Raffinose
- N-acetyl-D-glucosamine

Phosphate donors

The following phosphate donors may be used:

Substrate	K_m value [mM]	Relative rate
ATP*	0.1	1.0
dATP*	–	0.5
ITP	–	0.03
UTP*	–	0.004
CTP*, GTP*	–	0.001

Stabilizers

Thiols

Temperature Stability

The enzyme is relatively stable at temperatures <+40°C, but loses activity at temperatures >+40°C (pH 6.0, 10 minutes). At pH 6.0 to 8.0, Hexokinase (800 U/ml) in 0.1 M phosphate buffer does not lose activity in a 3 hour incubation at +15 to +25°C.

Unit Assay

For measuring Hexokinase activity, 0.22 M glucose in 0.1 M triethanolamine buffer, pH 7.6, 6.5 mM $MgCl_2$, 2.7 mM ATP, 0.83 mM NADP* is incubated in the presence of 1.7 U glucose-6-phosphate dehydrogenase* with an appropriate amount of Hexokinase (10 to 20 mU) at +25°C (total volume: 3.0 ml). The enzyme activity is calculated from the increase of absorbance at 340 nm (ε = 6.3 [l × mmol/l^{-1} × cm^{-1}]) or 365 nm (ε = 3.5 [l × mmol/l^{-1} × cm^{-1}]).

3. Additional Information on this Product

3.1. Test Principle

Reaction mechanism

Hexokinase catalyzes the reaction:

Hexose + ATP ↔ Hexose-6-phosphate + ADP

The relative rates and K_m-values may differ depending on the different substrates used.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
ATP	5 g	10 127 523 001
	10 g	10 127 531 001
dATP	250 µl, 25 µmol, 100 mM 6,250 standard PCR assays of 20 µl each.	11 934 511 001
	1,250 µl, 125 µmol, 100 mM 31,250 standard PCR assays of 20 µl each.	11 969 013 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM 125,000 standard PCR assays of 20 µl each.	03 732 681 001
UTP	400 µl, 40 µmol, 100 mM	11 140 949 001
NADP	100 mg	10 128 031 001
	500 mg	10 128 040 001
	1 g	10 128 058 001
	5 g	10 240 354 001
GTP	250 mg	10 106 399 001
Glucose-6-Phosphate Dehydrogenase (G6P-DH)	1,000 U, 1 ml	10 165 875 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

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