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Hexokinase/Glucose-6- Phosphate Dehydrogenase (HK/G6P-DH) from yeast/*Leuconostoc*

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overproducer; ATP: D-hexose 6-phosphotransferase/D-glucose-6-phosphate: NADP
1-oxidoreductase

Cat. No. 10 127 825 001 15 mg
 5 ml

Cat. No. 10 737 275 001 30 mg
 10 ml

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Hexokinase/Glucose-6-Phosphate Dehydrogenase (HK/G6P-DH)	Suspension in 3.2 M ammonium sulfate solution, pH approximately 6.	10 127 825 001	1 vial, 15 mg, 5 ml
			10 737 275 001	1 vial, 30 mg, 10 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/Glucose-6-Phosphate Dehydrogenase (HK/G6P-DH)	Store at +2 to +8°C.

1.3. Application

Product Description

Source

Hexokinase: yeast

G6P-DH: *Leuconostoc mesenteroides* and recombinant in *E. coli*

2. How to Use this Product

2.1. Before you Begin

General Considerations

Additional information

- The optimal pH for the coupled HK/G6P-DH reactions is pH 7.6 to 7.7. HK/G6P-DH may be used in assays from pH 6.6 (creatine kinase) to pH 9.5 (D-sorbitol).
- Mg^{2+} is required in the HK reaction. For optimal activity, add sufficient Mg^{2+} (usually 2.5 to 4.0 mM) to activate HK, but do not add excess Mg^{2+} .
- Do not use high concentrations of phosphate buffer in assays with HK/G6P-DH. Phosphate inhibits G6P-DH; assays in the literature typically use 20 to 69 mM phosphate. Substitution of another buffer, such as triethanolamine for phosphate avoids the problem.
- Trichloroacetic acid (TCA) inhibits HK/G6P-DH.

⚠ Do not use TCA to deproteinize samples to be assayed with these enzymes. Use perchloric acid instead.

2.2. Parameters

Absorbance

Absorbance of the purified G6P-DH enzyme

1.15 (1 mg enzyme/ml, 280.5 nm)

Activator

Hexokinase

- Requires Mg^{2+}
- Catecholamines

Glucose-6-Phosphate-Dehydrogenase

- $HCO_3^- \leq 0.3$ M activates slightly.

EC-Number

EC 2.7.1.1/1.1.1.49

Inhibition

Hexokinase

- Glucose-6- phosphate (G6P) ($K_i = 9.1$ mM; pH 8.0 at +25°C).
- Lyxose
- Sorbose-1-phosphate
- 6-deoxy-6-fluoro-glucose
- EDTA
- Thiol blocking agents, such as Hg^{2+} and 4-chloromercuribenzoate.
- Polyphosphates

Glucose-6-Phosphate-Dehydrogenase

- Phosphate ($K_i = 50$ mM)
- Pyridoxal-5'-phosphate ($K_i = 0.004$ to 0.006 mM)
- Acetyl-CoA
- CoA
- NADPH is a competitive inhibitor of the NAD-dependent reaction.
- ATP is a competitive inhibitor of the reaction with either NAD or NADP.
- Mg^{2+} reverses inhibition by ATP

Isoelectric Point

Hexokinase

4.5 to 4.8

Glucose-6-Phosphate-DH

4.6

Molecular Weight

LG6P-DH: 110,000 Da, is a dimer.

pH Optimum

Hexokinase

7.6 to 9.0

Glucose-6-Phosphate-Dehydrogenase

7.0 to 8.5. Maximal activity at 7.8.

Specificity

Hexokinase

Hexokinase phosphorylates substrates with different rates (pH 7.5, +30°C).

i Hexokinase requires Mg^{2+} ($K_m = 2.6 \text{ mM}$) for activity.

Substrate	Relative rate [mM]	K_m value
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:

Phosphate donor	Relative reaction rate
ATP*	1.0 (K_m 0.1 mM)
dATP*	0.5
ITP*	0.03
UTP*	0.004
CTP*, GTP*	0.001

The enzyme shows a low rate of XTPase activity toward ATP, ITP, and GTP, which is increased in the presence of a non-phosphorylatable hexose, such as D-xylose.

3. Additional Information on this Product

Glucose-6-Phosphate Dehydrogenase

At pH 7.8, +25°C, G6P-DH from *Leuconostoc* (LG6P-DH) is highly specific for D-glucose-6-phosphate ($K_m = 36 \mu\text{M}$, NADP as coenzyme; $64 \mu\text{M}$, NAD as coenzyme), but will use either NADP ($K_m = 7.4 \mu\text{M}$; relative rate = 1.0) or NAD ($K_m = 115 \mu\text{M}$; relative rate = 1.8) as coenzyme.

LG6P-DH does not react with:

- Fructose-6-phosphate
- Fructose-1,6-biphosphate

LG6P-DG will oxidize 2-deoxy-glucose-6-phosphate with NADP, but not with NAD, as coenzyme. There is a slow reaction with D-glucose.

- Glucose-1-phosphate
- Ribose-1-phosphate

Stabilizers

Hexokinase

Thiols

Glucose-6-Phosphate-Dehydrogenase

None

Unit Definition

- One unit HK will phosphorylate $1 \mu\text{mol}$ of D-glucose in one minute at +25°C and pH 7.6.
- One unit G6P-DH will oxidize $1 \mu\text{mol}$ of glucose-6-phosphate in one minute at +25°C and pH 7.6.

 *The coupled assay produces $1 \mu\text{mol}$ of NADH per μmol of D-glucose phosphorylated.*

Volume Activity

- 340 U Hexokinase/ml at +25°C with glucose and ATP as the substrates.
- 170 U Glucose-6-Phosphate Dehydrogenase/ml at +25°C with glucose-6-phosphate as the substrate.

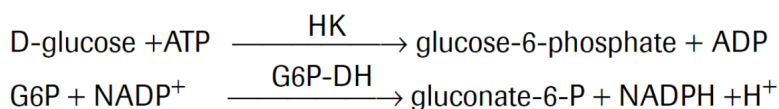
3. Additional Information on this Product

3.1. Test Principle

Preparation

Prepared by mixing Hexokinase with G6P-DH. Ratio of HK:G6P-DH is approximately 2:1 regarding protein content.

Control assay



Equilibrium

- With Hexokinase, the phosphorylation of glucose to glucose-6-phosphate is greatly favored at +30°C and pH 6.
- With G6P-DH, the oxidation (forward reaction) is strongly favored.



Turnover number G6P-DH

3.2×10^4 mol substrate/mol enzyme/minute with NADP as a coenzyme.

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	
 <i>Information Note: Additional information about the current topic or procedure.</i>	
 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.
1 2 3 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. Trademarks

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

4.4. License Disclaimer

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

4.5. Regulatory Disclaimer

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

4.6. Safety Data Sheet

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

4.7. 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

