Hexokinase/Glucose-6-Phosphate Dehydrogenase (HK/G6P-DH)

From yeast/leuconostoc overproducer ATP: D-hexose-6-phosphotransferase/D-glucose-6-phosphate: NADP-1-oxidoreductase EC 2.7.1.1/1.1.1.49

Cat. No. 10 127 825 001 15 mg (5 ml)
Cat. No. 10 737 275 001 30 mg (10 ml)

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

Product description

Form
Suspension in 3.2 M ammonium sulfate solution, pH approx. 6.

Preparation
Prepared by mixing hexokinase with G6P-DH. Ratio of HK:G6P-DH is approx. 2:1 regarding protein content.

Volume activity
340 U hexokinase/ml at 25°C with glucose and ATP as substrates. 170 U glucose-6-phosphate dehydrogenase/ml at 25°C with glucose-6-phosphate as substrate.

Storage and stability
The unopened solution is stable at +2 to +8°C until the expiration date printed on the label.

Handling instructions
- The optimal pH for the coupled HK/G6P-DH reactions is pH 7.6-7.7. However, HK/G6P-DH may be used in assays from pH 6.6 (creatine kinase) to pH 9.5 (D-sorbitol).
- Mg²⁺ is required in the HK reaction. For optimal activity, add sufficient Mg²⁺ (usually 2.5-4.0 mM) to activate HK, but do not add excess Mg²⁺.
- Do not use high concentrations of phosphate buffer in assays with HK/G6P-DH, since phosphate inhibits G6P-DH (assays in the literature typically use 20-69 mM phosphate.) Substitution of another buffer (e.g., 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.

Analysis Information
Roche quality control assay
D-glucose + ATP → glucose-6-phosphate + ADP
G6P + NADP⁺ → glucose-6-P + NADPH + H⁺

Unit definition
One unit (U) HK will phosphorylate 1 µmol of D-glucose in 1 min at +25°C and pH 7.6.
One unit (U) G6P-DH will oxidize 1 µmol of glucose-6-phosphate in 1 min at 25°C and pH 7.8.
The coupled assay produces 1 µmol of NADH per µmol of D-glucose phosphorylated.

Hexokinase

Source
From yeast

Equilibrium
The phosphorylation of glucose to glucose-6-phosphate is greatly favored at 30°C and pH 6.0.

Relative reaction rates
Hexokinase phosphorylates the following substrates (pH 7.5); 30°C

Note: HK requires Mg²⁺ (Km = 2.6 mM) for activity.

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

Note: HK does not phosphorylate: L-arabinose, D-xyllose, D-lyxose, L-rhamnose, D-galactose, sucrose, lactose, maltose, trehalose, raffinose and N-acetyl-D-glucosamine.
Phosphate donor’s

Please refer to the following table.

<table>
<thead>
<tr>
<th>Phosphate donor</th>
<th>Relative reaction rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>1.0 (Km 0.1 mM)</td>
</tr>
<tr>
<td>dATP</td>
<td>0.5</td>
</tr>
<tr>
<td>ITP</td>
<td>0.03</td>
</tr>
<tr>
<td>UTP</td>
<td>0.004</td>
</tr>
<tr>
<td>GTP</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Other activities

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.

Glucose-6-Phosphate Dehydrogenase (G6P-DH)

Source

D-Glucose-6-phosphate: NAD(P)+ 1-oxidoreductase, EC 1.1.1.49
from Leuconostoc mesenteroides and recombinant in E. coli

Equilibrium constant

The oxidation (forward reaction) is strongly favored.

Specificity, Km and relative reaction rates

(pH 7.8; +25°C) G6P-DH from Leuconostoc (LG6P-DH) is highly specific for D-glucose-6-phosphate (Km = 36 µM, NADP as coenzyme; 64 µM, NAD as coenzyme), but will use either NADP (Km = 74 µM; relative rate = 1.0) or NAD (Km = 115 µM; relative rate = 1.8) as coenzyme.

LG6P-DH does not react with
- fructose-6-phosphate
- fructose-1,6-biphosphate

Note: 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.

Enzyme structure and Mr

LG6P-DH (Mr ≥ 110,000) is a dimer

Absorbance of purified enzyme

1.15 (1 mg enzyme/ml, 280.5 nm)

Turnover number

3.2 × 10^4 mol substrate/mol enzyme/min (NADP as a coenzyme)

References

5 See reference 1.
6 See reference 1.
7 See reference 1.
8 See reference 1.
9 See reference 1.
10 See reference 1.
11 See reference 1.
12 See reference 1.
13 See reference 1.
14 See reference 1.
15 See reference 1.
16 See reference 1.
17 See reference 1.

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