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)

Store at +2 to +8°C

**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).
- Mg$^{2+}$ is required in the HK reaction. For optimal activity, add sufficient Mg$^{2+}$ (usually 2.5-4.0 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, 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

**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 7.6.
The coupled assay produces 1 µmol of NADH per µmol of D-glucose phosphorylated.

**Specificities**

- **Parameter**
  - **HK**
  - **G6P-DH**

- **pH**
  - 4.5-4.8
  - 4.6

- **pH optimum**
  - 7.6-9.0
  - 7.0-8.5 (maximal activity at 7.8)

- **Activators**
  - Mg$^{2+}$
  - catecholsamines
  - HCO$_3^-$ (<0.3 M) activates slightly

- **Stabilizers**
  - Thiols

- **Inhibitors**
  - glucose-6-phosphate (G6P) ($K_I = 9.1$ mM; pH 8.0; 25°C)
  - K$_II$ = 0.004-0.006 mM
  - acetyl-CoA
  - ATP is a competitive inhibitor of the NAD-dependent reaction

**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 Mg$^{2+}$ ($K_m = 2.6$ mM) for activity.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative rate</th>
<th>$K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucose</td>
<td>0.1 mM</td>
<td>1.0</td>
</tr>
<tr>
<td>D-fructose</td>
<td>0.7 mM</td>
<td>1.8</td>
</tr>
<tr>
<td>D-mannose</td>
<td>0.05 mM</td>
<td>0.8</td>
</tr>
<tr>
<td>D-glucosamine</td>
<td>1.5 mM</td>
<td>0.7</td>
</tr>
<tr>
<td>2-deoxy-D-glucose</td>
<td>0.3 mM</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Note:** HK does not phosphorylate: L-arabinose, D-xyllose, D-lxose, L-xyllose, 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>CTP</td>
<td>0.001</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-DH 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 × 10⁴ mol substrate/mol enzyme/min (NADP as a coenzyme)

**References**

3. Beach, FL.
5. See reference 1.
7. See reference 1.
15. See reference 1.

**Regulatory Disclaimer**

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**Changes to previous version**

Editorial changes.

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