

SPECIFICATION SHEET

Product Name: Hexokinase
Catalog # HK01D-E311H

Product Description: Recombinant research grade hexokinase from a microorganism. The enzyme is sold as a lyophilized white powder.

EC 2.7.1.1

Storage and Stability: Product can be stored at 2~8°C for transportation process up to ten days but long-term storage should be at -20°C.

Alternative Names	: D-Hexose-6-phosphotransferase
Unit Definition	: One unit causes the oxidation of one micromole of NADH per minute at 30°C, pH 8.5
Appearance	: White amorphous powder, lyophilized
Molecular Weight	: 55.1 kDa
Activity	: ≥500U / ≥1000U
Pack Size	: 500U / 1000U
Scientific Background	: Hexokinases phosphorylate hexoses to form hexose phosphate ¹ . The preferred substrate of these ubiquitous enzymes is D-glucose, but they can also phosphorylate other hexoses to varying degrees ² . Hexokinase is used in diagnostic tests to estimate the plasma glucose concentration of serum or plasma samples ³ . In the hexokinase method of plasma glucose estimation, glucose in the sample first reacts with adenosine triphosphate (ATP) with the help of the hexokinase enzyme to form glucose-6-phosphate. Glucose-6-phosphate is then acted upon by glucose-6-phosphate dehydrogenase (G6PD) in the presence of NADP ⁺ or NAD ⁺ to form NADPH or NADH and 6-phosphogluconate. The amount of NADPH or NADH generated is measured by recording absorbance at 340 nm, which is proportional to the glucose concentration in the sample.
Reference	1. Ciscato F, Ferrone L, Masgras I, Laquatra C, Rasola A. Hexokinase 2 in Cancer: A Prima Donna Playing Multiple Characters. <i>Int J Mol Sci.</i> 2021 Apr 29;22(9):4716. PMID: 33946854; PMCID: PMC8125560. 2. Cárdenas ML, Cornish-Bowden A, Ureta T. Evolution and regulatory role of the hexokinases. <i>Biochim Biophys Acta.</i> 1998 Mar 5;1401(3):242-64. PMID: 9540816. 3. Ambade VN, Sharma YV, Somani BL. METHODS FOR ESTIMATION OF BLOOD GLUCOSE: A COMPARATIVE EVALUATION. <i>Med J Armed Forces India.</i> 1998 Apr;54(2):131-133.

Hexokinase Activity Assay Protocol

Reaction Components

- 0.1 M Tris-HCl, pH 8.5
- 1 M D-(+)-Glucose solution, Sigma G8270 (prepared fresh in ddH₂O at least 1 hour at RT before use)
- 50 mM ATP solution (prepared fresh in ddH₂O)
- 30 mM NAD⁺ solution, Carbosynth NN11969 (prepared fresh in ddH₂O)
- 600 U/mL G-6-PDH solution (if stock solution needs to be diluted, use 0.1 M Tris-HCl (pH 8.5))
- 200 mM MgCl₂-6H₂O solution
- 50 mM Enzyme Dilution Buffer (50 mM Tris-HCl buffer, pH 7.5, added 0.1% BSA)

- Step 1** Reconstitute lyophilized enzyme powder in water to desired concentration.
- Step 2** Dilute reconstituted enzyme to 0.1-0.4 U/mL using enzyme diluent (G) immediately before the assay.
- Step 3** Prepare the following reaction mixture in a cuvette (d=1.0 cm) and equilibrate at 30°C for about 5 minutes.

1.5 mL Tris-HCl, pH 8.5 (A)

0.2 mL	Glucose solution	(B)
0.1 mL	ATP solution	(C)
0.1 mL	NAD ⁺ solution	(D)
0.2 mL	MgCl ₂ -6H ₂ O	(E)
0.01 mL	G-6-PDH solution	(F)
0.9 mL	ddH ₂ O	

Step 4 Add 0.1 mL of the enzyme solution to the reaction mixture and mix gently.

Step 5 Record the increase of optical density at 340 nm for 5 minutes in a spectrophotometer. Keep the reaction at a temperature of 30°C.

Note: Blank the spectrophotometer with water

Step 6 Calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test).

Step 7 Measure the blank rate (ΔOD blank) by the same method as the test except that the enzyme diluent (G) is added instead of the enzyme solution.

Unit definition: One unit causes the formation of one micromole of NADH per minute under the conditions described above.

Calculation: Activity can be calculated by using the following formula:

$$\text{Volume Activity (U/mL)} = \frac{\Delta\text{OD}/\text{min} (\Delta\text{OD test} - \Delta\text{OD blank}) \times V_t \times \text{df}}{(6.22 \times 1.0 \times V_s)} = \Delta\text{OD}/\text{min} \times 5.0 \times \text{df}$$

$$\text{Weight Activity (U/mg)} = (\text{U/mL}) \times 1/\text{C}$$

V_t: Total volume (3.11 mL)

V_s: Sample volume (0.1 mL)

6.22: Millimolar extinction coefficient of NADH (cm²/micromole)

df: Dilution factor

C: Enzyme concentration (mg/mL)