Catalog #	Aliquot Size
HK01D-E311H-500	500 U
HK01D-E311H-1000	1000 U

Hexokinase

Catalog # HK01D-E311H
Lot # A4616-10

Product Description

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

CAS #: 9001-51-8
Enzyme Commission #: 2.7.1.1
Molecular Weight: 55.1 kDa

Alternative name (s)

D-Hexose-6-phosphotransferase.

Storage and Stability

Store at –20°C. Once reconstituted, aliquot product into smaller quantities to avoid repeated handling and multiple freeze/thaw cycles and store at -70°C.

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.

References

1. Ciscato F, Ferrone L, Masgras I, Laquatra C, Rasola A. Hexokinase 2 in Cancer: A Prima Donna Playing Multiple Characters. Int J Mol Sci. 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. Biochim Biophys Acta. 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. Med J Armed Forces India. 1998 Apr;54(2):131-133.

Activity

Pack Size	Activity
500 U	≥500 U
1000 U	≥1000 U

Unit Definition: One unit causes the oxidation of one micromole of NADH per minute at 30°C pH 8.5.

Enzyme activity was determined using the Hexokinase Activity Assay Protocol provided below.

Hexokinase

Catalog #	HK01D-E311H
Lot #	*A4616-10*
Stability	1yr at –20°C from date of shipment
Storage & Shipping	Store at –20°C. Once reconstituted, aliquot product into smaller quantities to avoid repeated handling and multiple freeze/thaw cycles and store at -70°C. Product shipped on ice pack.

To place your order, please contact us by phone 1-778-326-0223 or 1-888-606-3424 (Toll free)
or by email: orders@signalchemdx.com or info@signalchemdx.com - www.signalchemdx.com

Hexokinase Activity Assay Protocol

Reaction Components

(A) 0.1M Tris-HCl, pH 8.5
(B) 1M D-(+)-Glucose solution, Sigma G8270 (prepared fresh in ddH2O at least 1 hour at RT before use)
(C) 50mM ATP solution (prepared fresh in ddH2O)
(D) 30mM NAD+ solution, Carbosynth NN11969 (prepared fresh in ddH2O)
(E) 600U/mL G-6-PDH solution (if stock solution needs to be diluted, use 0.1M Tris-HCl (pH 8.5))
(F) 200mM MgCl2-6H2O solution
(G) 50mM Enzyme Dilution Buffer (50mM Tris-HCl buffer, pH7.5, added 0.1% BSA)

Assay Protocol

Step 1. Reconstitute lyophilized enzyme powder in water to desired concentration.

Step 2. Dilute reconstituted enzyme to 0.1-0.4U/ml using enzyme diluent (G) immediately before the assay.

Step 3. Prepare the following reaction mixture in a cuvette (d=1.0cm) and equilibrate at 30°C for about 5 minutes

1.5ml	Tris-HCl, pH 8.5	(A)
0.2ml	Glucose solution	(B)
0.1ml	ATP solution	(C)
0.1ml	NAD+ solution	(D)
0.2ml	MgCl2-6H2O	(E)
0.01ml	G-6-PDH solution	(F)
0.9mL	ddH2O	

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

Step 5. Record the increase of optical density at 340nm 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:

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

Weight Activity (U/mg) = (U/mL) x 1/C

Vt: Total volume (3.11ml)

Vs: Sample volume (0.1mL)

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

df: Dilution factor

c: Enzyme concentration (mg/mL)

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: Hexokinase **Catalog # HK01D-E311H**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Supplier of Datasheet:	SignalChem Diagnostics Inc.
Street Address:	*190-13160 Vanier Place*
City, Prov. Postal Code:	*Richmond, BC, V6V 2J2*
Country:	*Canada*
Emergency Phone:	1-888-606-3424 (Toll free) 1-778-326-0223 (local)

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Not GHS classified.
- **Hazard Pictograms:** No labelling applicable.
- **Signal words:** None.
- **Hazard statements:** None.
- **Precautionary statements:** None.
- **Other hazards:** None known.

Article 3 – Composition/Information on Ingredients

Chemical Characterization: Mixture.
Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Sodium azide	NaN_3	26628-22-8	0.05%
EDTA disodium salt dihydrate	Ethylenediaminetetraacetic acid disodium salt-2-hydrate	6381-92-6	0.0372%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breath in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Remove contact lenses, if present and easy to do so. Consult a physician.
- **After swallowing:** Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

SAFETY DATA SHEET

Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment, and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
  NA
- **Appropriate engineering controls:**
  Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**

Respiratory protection:
Use appropriate respirator if there is inadequate ventilation by following the government standards.

Hand protection:
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.

Eye/face protection:
Safety goggles with side-shields approved under appropriate government standards.

Skin/body protection:
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Appearance: White powder (lyophilized)	**Danger of explosion:** Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	**Explosion limits:** Not available.
pH: Not available.	**Decomposition temperature:** Not available.
Melting point/freezing point: Not determined.	**Vapor pressure at 20 °C:** Not available.
Boiling point/Boiling range: Not determined.	**Density:** Not determined.
Flash point: Not determined.	**Relative density:** Not determined.
Flammability (solid, gaseous): Not determined.	**Vapor density:** Not determined.
Ignition temperature: Not determined.	**Evaporation rate:** Not determined.
Auto-igniting: Product is not self-igniting.	**Solubility in / Miscibility with Water:** Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Not determined.
- **Hazardous decomposition products:** Not determined.

SAFETY DATA SHEET

Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
  Inhalation: No data available
  Ingestion: No data available
  Skin: No data available
  Eyes: No data available
- **Signs and Symptoms of Exposure:** No data available
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** No data available.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 – Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.