Recombinant Human Hexokinase 1

Catalog Number: 8178-HK

DESCRIPTION

Source	*E. coli*-derived Phe11-Ser917, with an N-terminal Met and 6-His tag Accession # P19367
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	102 kDa

SPECIFICATIONS

SDS-PAGE	95-115 kDa, reducing conditions
Activity	Measured by its ability to phosphorylate glucose. The specific activity is >700 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, DTT, Glucose and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Universal Kinase Activity Kit (Catalog # EA004)
- 10X Assay Buffer (supplied in kit): 250 mM HEPES, 1500 mM NaCl, 100 mM $MgCl_2$, 100 mM $CaCl_2$, pH 7.0
- Recombinant Human Hexokinase 1 (rhHK-1) (Catalog # 8178-HK)
- Glucose (Sigma, Catalog # G5767), 100 mM stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare 1X Assay Buffer by diluting 10X Assay Buffer with deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Universal Kinase Activity Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock (this is the first standard curve dilution).
3. Prepare standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
4. Load 50 µL of each dilution of the standard curve into a plate in triplicate. Include a curve blank containing 50 µL of 1X Assay Buffer.
5. Prepare Substrate Mixture composed of 0.5 mM ATP and 25 mM Glucose in 1X Assay Buffer.
6. Dilute rhHK-1 to 7.5 ng/µL in 1X Assay Buffer.
7. Load 20 µL of the 7.5 ng/µL rhHK-1 into the plate in triplicate. Include a Control containing 20 µL of 1X Assay Buffer.
8. Dilute Coupling Phosphatase 4 (supplied in kit) to 10 µg/mL in 1X Assay Buffer.
9. Add 10 µL of 10 µg/mL Coupling Phosphatase 4 to wells containing enzyme and Control, excluding the standard curve.
10. Add 20 µL of Substrate Mixture to the wells, excluding the standard curve.
11. Incubate sealed plate at room temperature for 10 minutes.
12. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
13. Add 100 µL of deionized water to all wells. Mix briefly.
14. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
15. Read plate at 620 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg) x coupling rate**}}$$

*Derived from the phosphate standard curve using linear fitting and adjusted for Control.

**The coupling rate is 0.475 under these conditions.

Final Assay Conditions

Per Reaction:

- rhHK-1: 0.15 µg
- Coupling Phosphatase 4: 0.1 µg
- ATP: 0.2 mM
- Glucose: 10 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

Hexokinases phosphorylate hexose to form hexose 6-phosphate, the first step in hexose metabolism (1). Phosphorylation of a hexose adds charge to molecule thereby making it difficult to transport out of a cell. The hexose is therefore retained for intracellular metabolic processes, such as glycolysis or glycogen synthesis. In most organisms, glucose is the most important substrate of hexokinases and glucose-6-phosphate is the most important product. There are four mammalian hexokinases (2). Hexokinase 1, 2 and 3 are referred to as high-affinity hexokinases because their Km for glucose is below 1 mM. Hexokinase 4 is specific for glucose and is also referred to as glucokinase (3). Hexokinase I localizes to the outer membrane of mitochondria and is found in all mammalian tissues. The amino acids involved in mitochondrion membrane localization (4) have been removed in the recombinant enzyme. Hexokinase 1 (HK1) contains two homologous halves that are believed to be evolved from a single ancestral hexokinase by gene duplication and fusion (5). While the regulatory function is associated with the N-terminal half, the catalytic site is associated with the C-terminal half. HK1 is insensitive to product inhibition (6). Mutation in the active site of human hexokinase is associated with hexokinase deficiency and severe nonspherocytic hemolytic anemia (7). The enzymatic activity of recombinant human HK1 is measured using a phosphatase-coupled method (8).

References:

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