

**DESCRIPTION**

**Source** *E. coli*-derived human Hexokinase 2 protein  
Phe11-Arg917, with N-terminal Met and 6-His tag  
Accession # P52789

**N-terminal Sequence Analysis** Met

**Predicted Molecular Mass** 102 kDa

**SPECIFICATIONS**

**SDS-PAGE** 100-105 kDa, reducing conditions

**Activity** Measured by its ability to phosphorylate glucose.  
The specific activity is >1,000 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<sub>2</sub>, 100 mM CaCl<sub>2</sub>, pH 7.0
  - Recombinant Human Hexokinase 2 (rhHK-2) (Catalog # 8179-HK)
  - Glucose (Sigma, Catalog # G5767), 2 M 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 in 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 point of the standard curve.
  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 (supplied in kit) and 25 mM Glucose in 1X Assay Buffer.
  6. Dilute rhHK-2 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:

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

\*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-2: 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.

**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  $K_m$  for glucose is below 1 mM. Hexokinase 4 is specific for glucose and is also referred to as glucokinase (3). Hexokinase 2 (HK2), also known as muscle form hexokinase, localizes to the outer membrane of mitochondria and is present in adipose tissue, skeletal muscle, and heart (4). The amino acids corresponding to the mitochondrial binding domain (5) have been removed in the recombinant enzyme. Like HK1, HK2 contains two homologous halves that may have evolved from an ancestral hexokinase through gene duplication and tandem ligation (6). Unlike HK1, in HK2 both the C-terminal and N-terminal portions are catalytically active with the N-terminal half having higher activity than the C-terminal half (7). In HK2 both the N-terminal and C-terminal halves exhibit product inhibition. HK2 overexpression is required for tumor growth making HK2 an attractive oncotarget (4). The enzymatic activity of recombinant human HK2 is measured using a phosphatase-coupled method (8).

**References:**

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6. Ureta, T. (1982) *Comp Biochem Physiol. B.* **71B**:549.
7. Ahn, K.J. *et al.* (2009) *BMB Rep.* **42**:350.
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