EnzyChrom™ Hexokinase Assay Kit (EHXK-100)

Quantitative Colorimetric Kinetic Hexokinase Activity Determination

DESCRIPTION

HEXOKINASE (E.C. 2.7.1.1) is a mitochondrial and cytosolic enzyme and the first committed step in the glycolysis pathway. Hexose sugars, mainly Glucose, are phosphorylated using ATP to form Glucose-6-Phosphate. Hexokinases are implicated in a myriad of human diseases, including cancer and diabetes. BioAssay Systems' non-radioactive, colorimetric Hexokinase assay involves a stepwise reaction based on the reduction of the tetrazolium salt MTT in a NADPH-coupled enzymatic reaction to a colored product which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is proportional to the enzyme activity.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 0.22 to 100 U/L for 15 min reaction.

Convenient. Homogeneous "mix-incubate-measure" type assay.

High-throughput. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

Hexokinase activity determination in biological samples (e.g. microorganisms, plasma, serum, cultured cells, tissue and culture media.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Enzyme A:**	120 µL
NADP/MTT:	1 mL	**Enzyme B:**	120 µL
Substrate Mix:	1 mL	**Calibrator:**	1 mL

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

Sample Preparation: Serum and plasma are assayed directly.

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman or cell scraper. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation: Equilibrate reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge tubes before use.

Prepare enough Working Reagent (WR) for all assay wells by mixing, for each 96-well assay: 8 µL Substrate Mix, 8 µL NADP/MTT Solution, 1 µL Enzyme A, 1 µL Enzyme B and 70 µL Assay Buffer.

Reaction Preparation:

1. Transfer 100 µL H_2O (OD_{H2O}) and 100 µL Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 20 µL of each sample into separate wells and then add 80 µL WR to each sample well. Tap plate briefly to mix.
3. Read OD_{565nm} (OD_0), and again after 15 min (OD_{15}) on a plate reader.

CALCULATION

Subtract the OD_0 from OD_{15} for each sample to compute the ΔOD_S values. Hexokinase activity can then be calculated as follows:

$$\text{Hexokinase Activity} = \frac{\Delta OD_S}{\varepsilon_{mtt} \cdot l} \times \frac{\text{Reaction Vol (µL)}}{t\,(\text{min}) \cdot \text{Sample Vol (µL)}} \times n$$

$$= \frac{\Delta OD_S}{OD_{CAL} - OD_{H20}} \times \frac{273}{t\,(\text{min})} \times n \quad (U/L)$$

where ε_{mtt} is the molar absorption coefficient of reduced MTT. l is the light pathlength which is calculated from the calibrator. OD_{CAL} and OD_{H20} are OD_{565nm} (OD_o) values of the Calibrator and water. t is the reaction time (15 min is the recommended time). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. n is the dilution factor.

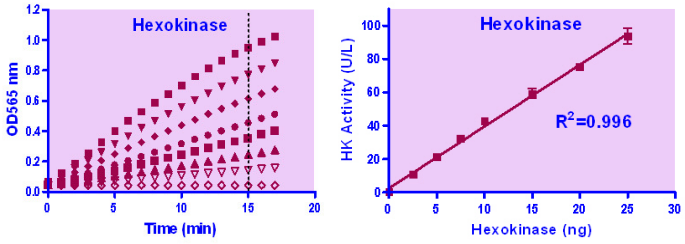
Unit definition: 1 Unit (U) of Hexokinase will catalyze the conversion of 1 µmole of Glucose to Glucose-6-Phosphate per min at pH 8.2.

Note: If sample Hexokinase activity exceeds 100 U/L, either use a shorter reaction time or dilute samples in buffer and repeat the assay. For samples with Hexokinase activity < 1 U/L, the incubation time can be extended to 2 hours.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

Hexokinase Titration Curves



Raw Kinetic Data ***Hexokinase Activity (15 min, 25 °C)***

LITERATURE

1. De Jesus, A et al (2022) Hexokinase 1 cellular localization regulates the metabolic fate of glucose. Mol. Cell. 82:1261-1277.
2. Patra, KC et al (2013) Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. Cancer Cell. 24: 213-228.
3. Wasserman, DH (2022) Insulin, Muscle Glucose Uptake, and Hexokinase: Revisiting the Road Not Taken. Physiology (Bethesda). 37: 115-127.