Hexokinase Microplate Assay Kit

Cat #: orb390779 (manual)

Detection and Quantification of Hexokinase (HK) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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INTRODUCTION

Hexokinases, which catalyze the ATP-dependent phosphorylation of aldo- and keto-hexoses to hexose-6-phosphate, catalyze the first step in a number of important biochemical pathways. In mammalian tissues, glucose is the predominant substrate for the hexokinases. There are four isozymes of hexokinase in mammalian tissue, (HK-I, -II, -III, and -IV), which differ in their kinetic and regulatory properties as well as tissue distribution and cofactor use. The hexokinases catalyze the first step in most of the relevant glucose metabolism pathways. Alterations in hexokinase activity are associated with multiple disorders such as X-linked muscular dystrophy, hemolytic anermias, and cancer.

Hexokinase Microplate Assay Kit provides a simple and direct procedure for measuring hexokinase activity in a variety of samples. Hexokinase activity is determined by a coupled enzyme assay, in which glucose is converted to glucose-6-phosphate by hexokinase, which is oxidized by glucose-6-phosphate dehydrogenase to form NADPH. The resulting NADPH reduces a colorless probe resulting in a colorimetric (340 nm) product proportional to the hexokinase activity present.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Enzyme	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Substrate: add 18 ml Reaction Buffer to dissolve before use.

Enzyme: add 1 ml Assay Buffer to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 μmol/L.

Positive Control: add 1 ml distilled water to dissolve before use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) ; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank	Positive Control
Standard	--	200 µl	--	--
Distilled water	--	--	200 µl	--
Enzyme	10 µl	--	--	10 µl
Substrate	180 µl	--	--	180 µl
Sample	10 µl	--	--	--
Positive Control	--	--	--	10 µl
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION

Unit Definition: One unit of HK activity is defined as the enzyme that generates 1 nmol of NADPH per minute.

1. According to the protein concentration of sample

$$HK\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$HK\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$HK\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$HK\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/ml;

$V_{Standard}$: the volume of standard, 200 μl = 0.2 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

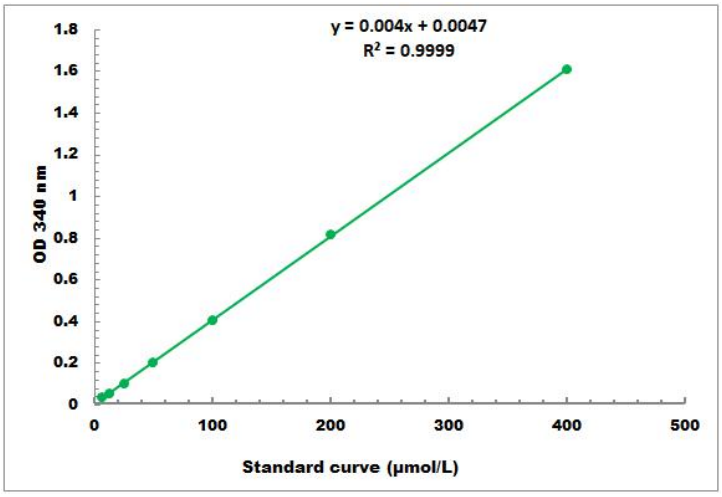
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

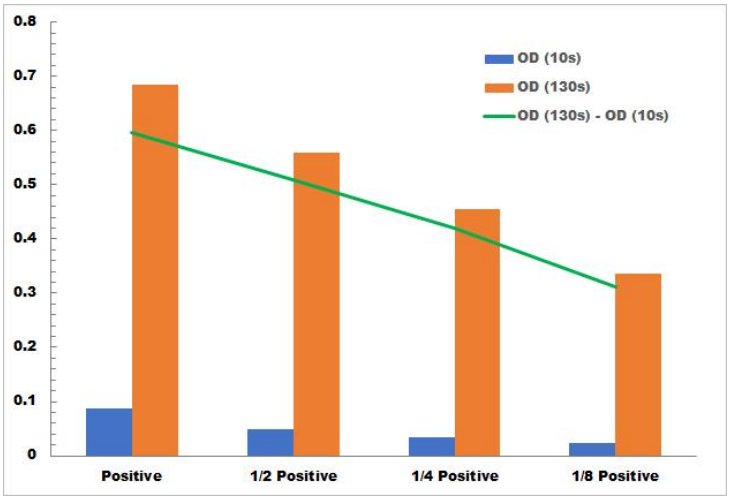
T: the reaction time, 2 minutes.

TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 µmol/L - 400 µmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

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