



Hexokinase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1073

(Version 1.5E)

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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I. 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 anemias, and cancer.

Hexokinase Activity Colorimetric 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 (450 nm) product proportional to the hexokinase activity present.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	20 mlx 1	4 °C
Substrate	Powderx 1	-20 °C
Enzyme	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 400 µmol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 µmol/L.

Substrate: Add 12 ml Reaction Buffer to dissolve before use. Keep in dark for immediate use or store at -20°C for 1-2 weeks after reconstitution.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use. Store at -80°C for 1 month after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use. Store at -80°C for 1 month after reconstitution.

Dye Reagent A: Briefly centrifuge prior to opening. Add 5 ml distilled water to dissolve before use, mix, store at 4°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Substrate	120 μ l	120 μ l	120 μ l	--	--
Sample	10 μ l		--	--	--
Distilled water	--	10 μ l	--	40 μ l	140 μ l
Positive Control	--	--	10 μ l	--	--
Enzyme	10 μ l	10 μ l	10 μ l	--	--
Mix and incubate at 37°C for 15 minutes.					
Standard	--	--	--	100 μ l	--
Dye Reagent A	50 μ l	50 μ l	50 μ l	50 μ l	50 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
Mix and incubate at room temperature for 2 minutes, record absorbance measured at 450 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

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

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10⁴

C_{Standard}: the concentration of standard, μmol/L

V_{Standard}: the volume of standard in assay procedure, mL

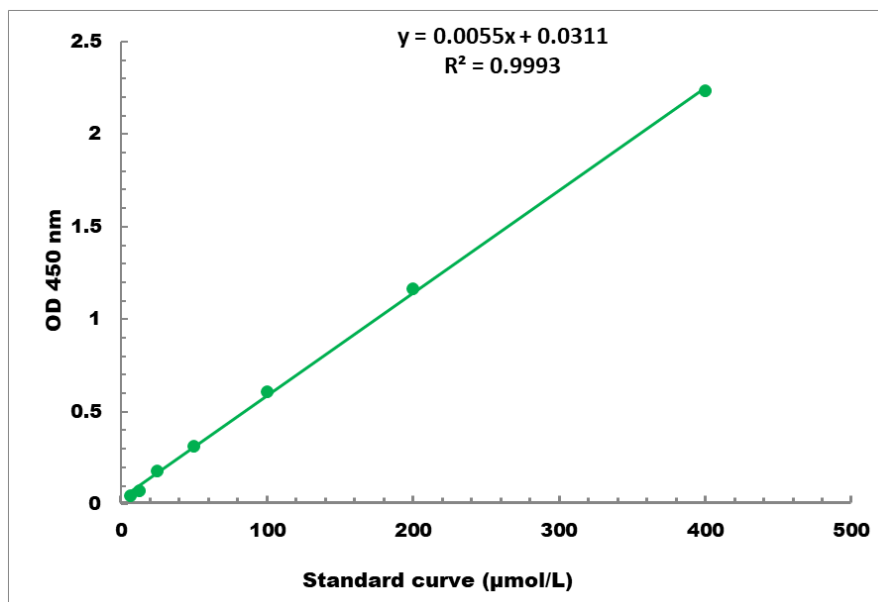
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

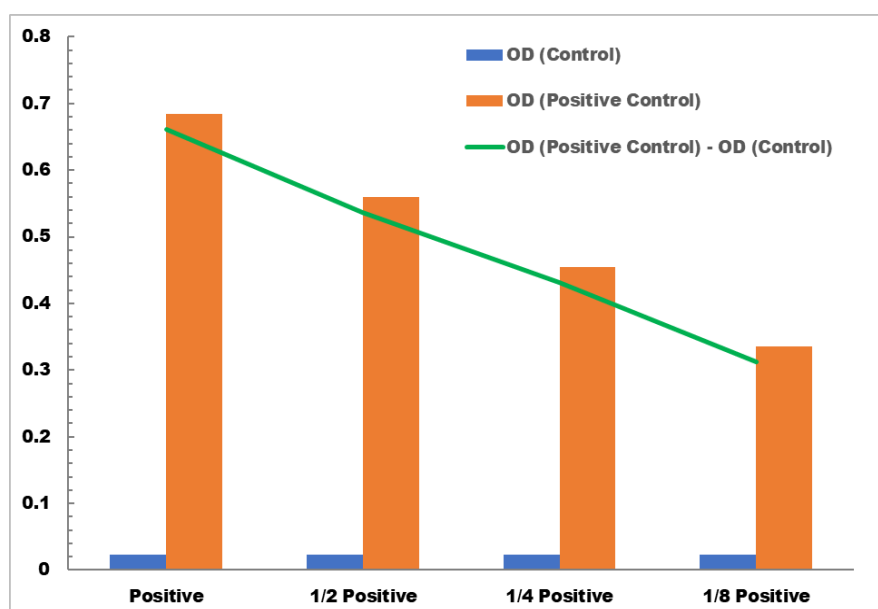
T: the reaction time, minute

VIII. 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