CheKine™ Micro Hexokinase (HK) Activity Assay Kit

Cat #: KTB1123 Size: 48 T/24 S 96 T/48 S

	Micro Hexokinase (HK) Activity Assay Kit		
REF	**Cat #:** KTB1123	LOT	**Lot #:** Refer to product label
	Detection range: 31.25-2,000 μM (The detection range of the standard should be converted into the detection range of activity according to the sample)		**Sensitivity:** 31.25 μM (The sensitivity of the standard should be converted into the sensitivity of the activity according to the sample)
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Culture Supernatant, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Hexokinase (HK, EC 2.7.1.1) is widely present in animals, plants, microorganisms and cultured cells. It is the first key enzyme in the process of glucose decomposition and catalyzes the conversion of glucose into glucose 6-phosphate, which is the intersection of glycolysis and pentose phosphate pathways. CheKine™ Micro Hexokinase (HK) Activity Assay Kit provides a convenient tool for sensitive detection of HK activity in Serum, Plasma, Animal and Plant Tissues, Cells, Cell Culture Supernatant, Bacteria and other samples. The principle is that HK catalyzes glucose synthesis glucose 6-phosphate, glucose 6-phosphate dehydrogenase further catalyzes the dehydrogenation of glucose 6-phosphate to form NADH. The formed NADH reduces a formazan (WST-8) reagent, which has a maximum absorption peak detected at about 450 nm. The enzyme activity was calculated by detecting the rate of increase in absorption at 450 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Assay Buffer	16 mL	32 mL	4℃
Substrate Mix	Powder×1 vial	Powder×1 vial	4℃, protected from light
Enzyme Mix	45 μL	90 μL	-20℃, protected from light
WST-8	400 μL	800 μL	-20℃, protected from light
Enhancer	80 μL	160 μL	-20℃, protected from light
NADH Standard	Powder×1 vial	Powder×2 vials	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Substrate Mix: Add 15.2 mL Assay Buffer for 48 T or 30.4 mL Assay Buffer for 96 T to dissolve before use. This solution can be stored at 4℃ for 1 week. The solution can also be stored at -20℃ for 1 month, protected from light after aliquoting to avoid repeated freezing and thawing.

Enzyme Mix: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

WST-8: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

Enhancer: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

NADH Standard: Add 1 mL deionized water to dissolve before use. The concentration is 2,000 μM, which could be stored at 4℃ for 1 week. The solution can also be stored at -20℃ for 1 month, protected from light after aliquoting to avoid repeated freezing and thawing.

Working Reagent: For each test well, prepare 190 μL Working Reagent for test by mixing 183 μL Substrate Mix, 1 μL Enzyme Mix, 5 μL WST-8, 1 μL Enhancer. Fresh reconstitution is recommended.

Working Reagent for control: For each control well, prepare 190 μL Working Reagent for control by mixing 184 μL Substrate Mix, 5 μL WST-8, 1 μL Enhancer. Fresh reconstitution is recommended.

Standard preparation:

Standard curve setting: dilute 2,000 μM standard with deionized water to 2,000, 1,000, 500, 250, 125, 62.5, 31.25 μM standard solution as shown in the table below.

Num.	Volume of Standard	Volume of Deionized Water (μL)	Concentration (μM)
Std.1	200 μL 2,000 μM	0	2,000
Std.2	100 μL of Std.1 (2,000 μM)	100	1,000
Std.3	100 μL of Std.2 (1,000 μM)	100	500
Std.4	100 μL of Std.3 (500 μM)	100	250
Std.5	100 μL of Std.4 (250 μM)	100	125
Std.6	100 μL of Std.5 (125 μM)	100	62.5
Std.7	100 μL of Std.6 (62.5 μM)	100	31.25

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal Tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.

2. Plant Tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20%, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.

3. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria in ice bath 5 min (power 20%, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.

4. Cell Culture Supernatant or Plasma, Serum: Tested directly.

Note: For animal tissues with high fat content, remove the upper layer of fat after centrifugation, and then take the supernatant. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheated the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm. Visible spectrophotometer was returned to zero with deionized water.

2. For each test well, add 10 μL sample and 190 μL Working Reagent for test per well, mix well. For each control well, add 10 μL sample and 190 μL Working Reagent for control per well in 96-well plate or microglass cuvette, mix well. Then incubate in room temperature 5 min. And reading the values at 450 nm. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Control}$.

3. For each standard well, add 10 μL diluted standard and 190 μL working reagent for test per well, mix well. For blank well, add 10 μL deionized water and 190 μL working reagent for test per well in 96-well plate or microglass cuvette, mix well. Then incubate in room temperature 5 min. And reading the values at 450 nm. Finally, calculate $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Each sample has a control well to eliminate the color of the sample itself. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.001, increase the sample quantity appropriately. If the sample's $\Delta A_{Test}>1.5$, please further dilute the sample with Extraction Buffer. Pay attention to multiply by the dilution factor when calculating the result. The animal and plant tissue samples extracted by this kit can also be used for the determination of KTB1122 and KTB1126.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (1 μM=1 nmol/mL). It's the NADH content.

2. Calculate the activity of HK in sample

(1) By protein concentration

Unit Definition: 1 nmol NADH produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

HK (U/mg prot)=y÷Cpr÷T×n**=0.2×y÷Cpr×n**

(2) Calculated by fresh weight of samples

Unit Definition: 1 nmol NADH produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

HK (U/g)=y÷W÷T×n**=0.2×y÷W×n**

(3) Calculated by bacteria or cell number

Unit Definition: 1 nmol NADH produced per min in 10^4 bacteria or cells reaction system is defined as a unit of enzyme activity.

HK ($U/10^4$)=y÷500÷T×n**=0.0004×y×n**

(4) Calculated by volume of liquid sample

Unit Definition: 1 nmol NADH produced per min in 1 mL liquid samples reaction system is defined as a unit of enzyme activity.

HK (U/mL)=y÷V_{Sample}÷T×n**=20×y×n**

Where: Cpr: Sample protein concentration, mg/mL; T: Reaction time, 5 min; n: Dilution factor; W: Sample weight, g; 500: Total number of bacteria or cells, 5×10^6; V_{Sample}: Sample volume added, 0.01 mL.

Typical Data

Typical standard curve:

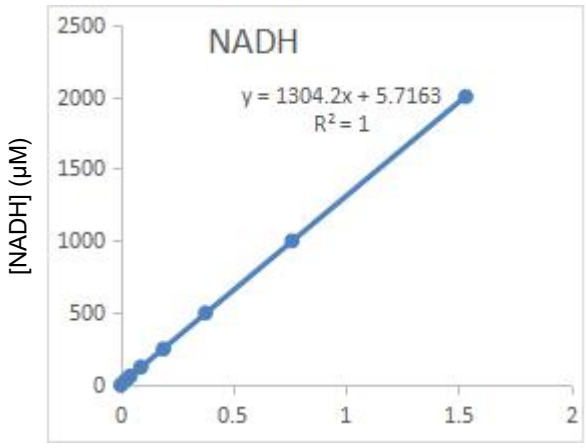

Figure 1. Standard curve of HK in 96-well plate assay-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Examples

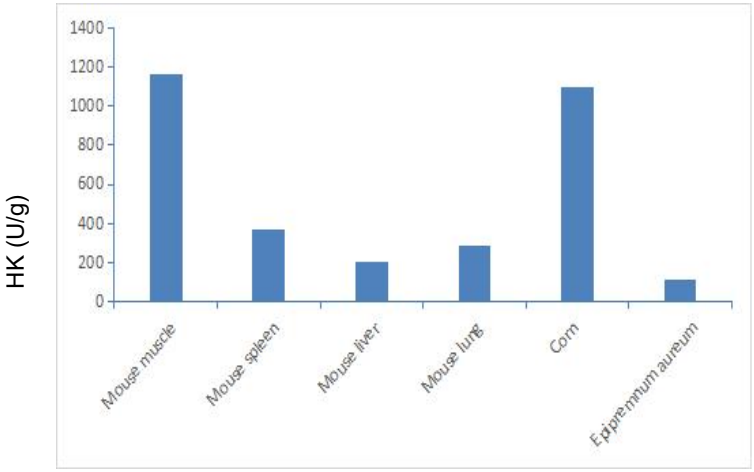

Figure 2. HK activity in mouse muscle, mouse spleen, mouse liver, mouse lung, corn and epipremnum aureum respectively. Assays were performed following kit protocol.

Recommended Products

Catalog No.	Product Name
KTB1120	CheKine™ Micro Pyruvate Kinase (PK) Assay Kit
KTB1122	CheKine™ Micro Phosphoenolpyruvate Carboxylase (PEPC) Activity Assay Kit
KTB1110	CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.