# CheKine™ Hexokinase (HK) Activity Assay Kit (UV-Vis)

Cat #: KTB1130 Size: 48 T/48 S 96 T/96 S

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| | **Hexokinase (HK) Activity Assay Kit (UV-Vis)** | | |
| REF | **Cat #:** KTB1130 | LOT | **Lot #:** Refer to product label |
| | **Applicable sample:** Animal and plant tissues, cells, bacteria, fungus, serum (plasma), cell culture | | |
| | **Storage:** Stored at -20℃ for 6 months, protected from light | | |

## Assay Principle

Hexokinase (Hexokinase, HK, EC2.7.1.1) is widely present in animals, plants, microorganisms and cultured cells. It is the first key enzyme in the decomposition process of glucose, catalyzing the conversion of glucose into glucose 6-phosphate, which is the intersection of glycolysis and pentose phosphate pathways. CheKine™ Hexokinase (HK) Activity Assay Kit (UV-Vis) can detect biological samples such as animal and plant tissues, cells, bacteria, fungus, serum, plasma or cell culture. In this kit, HK catalyzes the synthesis of glucose 6-phosphate glucose dehydrogenase further catalyzes the dehydrogenation of glucose 6-phosphate to form NADPH, which has a characteristic absorption peak at 340 nm.

## Materials Supplied and Storage Conditions

| Kit components | Size | | Storage conditions |
|---|---|---|---|
| | 48 T | 96 T | |
| Extraction Buffer | 60 mL | 60 mL×2 | 4℃ |
| Reagent Ⅰ | 14 mL | 28 mL | 4℃ |
| Reagent Ⅱ | Powder×1 vial | Powder×1 vial | -20℃, protected from light |
| Reagent Ⅲ | Powder×1 vial | Powder×1 vial | -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 ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV microplate or microquartz cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Water bath, freezing centrifuge
- Deionized Water, normal saline
- Mortar or homogenizer (for tissue samples)

## Reagent Preparation

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

**Reagent I:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

**Working Reagent II:** Prepared before use. Add 10.8 mL Reagent Ⅰ to Reagent Ⅱ for 48 T, and 21.6 mL Reagent Ⅰ to Reagent Ⅱ for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

**Working Reagent III:** Prepared before use. Add 0.5 mL Reagent Ⅰ to Reagent Ⅲ for 48 T, and 1 mL Reagent Ⅰ to Reagent Ⅲ for 96 T to fully dissolve. According to the dosage, dilute the dissolved Reagent Ⅲ 10 times with Reagent Ⅰ to Working Reagent Ⅲ. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

**Working Reagent:** Prepared before use. According to dosage, prepare according to the ratio of Working Reagent Ⅱ: Working Reagent Ⅲ=180: 10. Working Reagent is freshly prepared.

## Sample Preparation

**Note: We recommend that you use fresh samples. HK activity in frozen samples will be significantly reduced**

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells, Bacteria or Fungus : Collect $5\times10^6$ cells, bacteria or fungus into the EP tube, wash cells, bacteria or fungus with cold PBS, centrifuge at 800 g for 2 min and discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells, bacteria or fungus 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma or other Liquid samples: Test directly.

**Note: 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. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Preheated Working Reagent for 10 min at 37℃ bath.
3. Operation table (The following operations are operated in the 96-well UV plate or microquartz cuvette):

| Reagent | Test Well (μL) |
|---|---|
| Sample | 10 |
| Working Reagent | 190 |

4. After adding Working Reagent, immediately mix well, measure the absorbance value $A_1$ at 10 s at 340 nm, and the absorbance value $A_2$ at 310 s at 37℃ for 10 min. Finally calculate $\Delta A=A_2-A_1$.

**Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.005, increase the sample quantity appropriately, or increase the reaction time (15-30 min). If ΔA is greater than 0.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the reaction time to 5 min.**

## 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.**

Calculation of HK activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: The production of 1 nmol of NADPH per milligram of protein per min was defined as one unit of enzyme activity.

HK (U/mg prot)=[ΔA×$V_{Total}$÷(ε×d)×$10^9$]÷($V_{Sample}$×Cpr)÷T×n**=643.09×ΔA÷Cpr×n**

(2) Calculated by fresh weight of samples

Active unit definition: The production of 1 nmol of NADPH per gram tissue per min was defined as one unit of enzyme activity.

HK (U/g fresh weight)=[ΔA×$V_{Total}$÷(ε×d)×$10^9$]÷(W×$V_{Sample}$÷$V_{Total\ sample}$)÷T×n**=643.09×ΔA÷W×n**

(3) Calculated by cells, bacteria or fungus

Active unit definition: The production of 1 nmol of NADPH per $10^4$ cells, bacteria or fungus per min was defined as one unit of enzyme activity.

HK (U/$10^4$)=[ΔA×$V_{Total}$÷(ε×d)×$10^9$]÷(500×$V_{Sample}$÷$V_{Total\ sample}$)÷T×n**=643.09×ΔA÷500×n**

(4) Calculated by volume of liquid samples

Active unit definition: The production of 1 nmol of NADPH per mL liquid was defined as one unit of enzyme activity.

HK (U/mL)=[ΔA×$V_{Total}$÷(ε×d)×$10^9$]÷($V_{Sample}$÷$V_{Total\ sample}$)÷T×n**=643.09×ΔA×n**

ε: NADPH molar extinction coefficient, 6.22×$10^3$ L/mol /cm; d: the light path of the 96-well plate, 0.5 cm; $10^9$: 1 mol=1×$10^9$ nmol; $V_{Total}$: total reaction volume, 200 μL=2×$10^{-4}$ L; $V_{Sample}$: sample volume added, 10 μL=1×$10^{-5}$ L; $V_{Total\ sample}$: added Extraction Buffer volume , 1 mL; Cpr: sample protein concentration, mg/mL; W: weight of sample,g; T: reaction time, 10 min; 500: Number of cells, bacteria or fungus, 5×$10^6$, calculated in units of $10^4$; n: Sample dilution multiple.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

## Typical Data

Example-1: Take 0.1 g of fresh mouse leg muscle, follow the measurement steps, and use a 96-well UV plate for detection. Measured ΔA=$A_2$-$A_1$=0.5997-0.1189=0.4808. Calculated according to the sample quality, HK (U/g fresh weight)=643.09×ΔA÷W×n=3,091.98 U/g.

## Recommended Products

| Catalog No. | Product Name |
|---|---|
| KTB1010 | CheKine™ Micro Coenzyme II NADP(H) Assay Kit |
| KTB1600 | CheKine™ Micro Reduced Glutathione (GSH) Assay Kit |
| KTB1610 | CheKine™ Micro Glutathione Oxidized (GSSG) 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.