SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

# Hexokinase (HK) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/ microplate reader

**Catalog Number:** AK0515

**Size:** 100T/96S

**Components:**

Extract solution:100 mL×1 bottle, Storage at 4℃ .

Reagent I:20 mL×1. Storage at 4℃ .

Reagent II: Powder×1. Storage at 4℃ . Before use, add 18 mL of Reagent Ⅰ to dissolve completely, and put it in water bath at 37℃ (mammalian) or 25℃ (other species) for 5 min; store the unused reagent at 4℃ for one week.

Reagent III: Powder×2. Storage at -20℃ . Before use, take one and add 0.5 mL of Reagent Ⅰ to fully dissolve it for use; keep the unused reagent at 4℃ for one week.

**Product Description:**

Hexokinase (HK, EC 2.7.1.1) is widely distributed in animals, plants, microorganisms and cultured cells. It is the first key enzyme in the process of glucose decomposition, catalyzing the conversion of glucose into glucose 6-phosphate, which is the intersection of glycolysis and pentose phosphate pathways.

HK catalyzes the synthesis of glucose to 6-phosphate glucose, and 6-phosphate glucose dehydrogenase further catalyzes the dehydrogenation of 6-phosphate glucose to NADPH, which has a characteristic absorption peak at 340 nm.

**Reagents and Equipment Required but Not Provided:**

Spectrophotometer/microplate reader, water-bath, table centrifuge, adjustable pipette, micro quartz cuvette/96 well flat-bottom plate (UV plate), mortar/homogenizer and ice.

**Procedure:**

**I. Sample preparation:**

a. Bacteria or cultured cells: Collecting bacteria or cells to the centrifugal tube, discard the supernatant after the centrifuge. The number of bacteria or cells ($10^4$): Extract solution volume (mL) is 500~1000:1 (It is suggested that add 1 mL Extract solution to 5 million bacteria or cells). Ultrasonic to break bacteria or cells (20% or 200 W on ice bath, ultrasound for 3 s, interval of 10 s, repeat 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

b. Tissues: The tissues mass (g): Extract solution volume (mL) is 1:5~10 (it is suggested that add 1 mL Extract solution to about 0. 1g tissues). and homogenize in ice bath; Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

c. Serum (plasma) sample: direct detection.

**II. Measurement operation:**

a. Preheat spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 340 nm and set zero with distilled water.

b. Sample Test:

Add 180 μL of Reagent II, 10 μL of Reagent III and 10 μL of sample into micro quartz cuvette/96-well flat-bottom plates, mix thoroughly, immediately record absorbance value A1 at 340 nm of 20s. after colorimetric, rapidly place the micro quartz cuvette/ enzyme plate together with the reaction solution in 37℃(mammals) or 25℃ (other species) water-bath/incubator. react accurately for 5 minutes; Quickly take the micro quartz cuvette and wipe dry it, and measure absorbance at 340 nm and record absorbance A2 of 320 seconds, calculate ΔA = A2 - A1.

**III. Calculation of HK activity calculation:**

**a. Calculate by micro cuvette:**

1. Calculation of serum (plasma) HK activity:

Definition of unit: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every milliliter of serum (plasma).

$HK(U/mL) = [\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9] \div V_S \div T = 643 \times \Delta A$

2. Calculation of HK activity in tissue, bacteria or cells:

1) Calculate by sample protein concentration

Definition of unit: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every milligram of sample protein.

$HK(U/mg\ prot) = [\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9] \div (V_S \times Cpr) \div T = 643 \times \Delta A \div Cpr$

2) Calculate by sample fresh weight

Definition of unit: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every gram of sample.

$HK(U/g\ fresh\ weight) = [\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9] \div (W \times V_S \div V_{TS}) \div T = 643 \times \Delta A \div W$

3) Calculate by bacteria or cell density

Definition of unit: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every $10^4$ cells.

$HK(U/10^4\ cell) = [\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9] \div (500 \times V_S \div V_{TS}) \div T = 1.286 \times \Delta A$

$V_{TV}$ : Total volume of the reaction system, $2 \times 10^{-4}$ L;

ε: The molar extinction coefficient of NADPH,$6.22 \times 10^3$ L/mol/cm.

d: Light path of the cuvette, 1 cm;

$V_S$ : Add the sample volume, 0.01 mL;

$V_{TS}$ : Add extraction liquid volume, 1 mL;

T: Reaction time, 5 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

500: Total number of bacteria or cells, 5 million.

**b. Calculate by 96 well flat-bottom plate**

1. Calculation of serum (plasma) HK activity:

Definition of unit: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every milliliter of serum (plasma).

HK(U/mL) =[ΔA×$V_{TV}$÷(ε×d)×$10^9$]÷$V_S$÷T=1071.7×ΔA

2. Calculation of HK activity in tissue, bacteria or cells:

1) Calculate by sample protein concentration

Definition of unit: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every milligram of sample protein.

HK(U/mg prot) =[ΔA×$V_{TV}$÷(ε×d)×$10^9$]÷($V_S$ ×Cpr) ÷T=1071.7×ΔA÷Cpr

2) Calculate by sample fresh weight

Definition of unit: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every gram of sample.

HK(U/g fresh weight) =[ΔA×$V_{TV}$÷(ε×d)×$10^9$]÷(W ×$V_S$÷$V_{TS}$)÷T=1071.7×ΔA÷W

3) Calculate by bacteria or cell density

Definition of unit: One unit of enzyme is defined as the amount of enzyme catalyzes the production of 1 nmol of NADPH per minute every $10^4$ cells.

HK(U/$10^4$ cell) =[ΔA×$V_{TV}$÷(ε×d)×$10^9$]÷(500×$V_S$÷$V_{TS}$)÷T=2. 143×ΔA

$V_{TV}$ : Total volume of the reaction system, $2×10^{-4}$ L;

ε: The molar extinction coefficient of NADPH is $6.22×10^3$ L/mol/cm.

d: Light path of the cuvette, 0.6 cm;

$V_S$ : Add the sample volume, 0.01 mL;

$V_{TS}$ : Add extraction liquid volume, 1 mL;

T: Reaction time, 5 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

500: Total number of bacteria or cells, 5 million.

**Note:**

1. The reaction solution in the cuvette must be kept at 37℃ or 25℃ . Take a small beaker and add a certain amount of 37℃ or 25℃ distilled water, put the beaker in 37℃ or 25℃ water-bath. In the reaction process, the cuvette and the reaction solution is placed in this beaker.

**2.** It is better for two people to do this experiment at the same time to ensure the accuracy of the experimental results. One for measuring the absorbance and the other timing.

**3.** If ΔA > 0.5, the tissue vitality is too high. In order to improve the detection sensitivity and make ΔA < 0.5, suggest the dilute the homogenate to a proper concentration by Extract solution (multiplied by the corresponding dilution factor in the calculation formula) or short the reaction time to 2 minutes

**Recent Product Citations:**

[1] Geng M T, Yao Y, Wang Y L, et al. Structure, expression, and functional analysis of the

hexokinase gene family in cassava[J]. International journal of molecular sciences, 2017, 18(5): 1041.

[2] Zhou F, Du J, Wang J. Albendazole inhibits HIF- 1α-dependent glycolysis and VEGF expression in non-small cell lung cancer cells[J]. Molecular and cellular biochemistry, 2017, 428(1-2): 171- 178.

[3] Liu Y, Liang X, Zhang G, et al. Galangin and pinocembrin from propolis ameliorate insulin resistance in HepG2 cells via regulating Akt/mTOR signaling[J]. Evidence-Based Complementary and Alternative Medicine, 2018, 2018.

[4] Jing Li,Yabing Duan,Chuanhong Bian,et al. Effects of validamycin in controlling Fusarium head blight caused by Fusarium graminearum: Inhibition of DON biosynthesis and induction of host resistance. Pesticide Biochemistry and Physiology. January 2019;153:152- 160.(IF2.87)

**References:**

[1] Pancera S M, Gliemann H, Schimmel T, et al. Adsorption behavior and activity of hexokinase[J]. Journal of colloid and interface science, 2006, 302(2): 417-423.

**Related Products:**

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|---|---|
| AK0540/AK0539 | Pyruvate Kinase(PK) Activity Assay Kit |
| AK0542/AK0541 | Phosphofructokinase(PFK) Activity Assay Kit |
| AK0247/AK0242 | Phosphoenolpyruvate Carboxylase(PEPC) Activity Assay Kit |