Hexokinase (HK) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC0745

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 25 mL×1	2-8℃
Reagent II A	Liquid 3 mL×1	2-8℃
Reagent II B	Powder×1	2-8℃
Reagent II C	Powder×1	-20℃
Reagent II D	Powder×1	-20℃
Reagent III	Powder×2	-20℃

Solution Preparation:

1. **Reagent II B:** Dissolve with 12 mL of Reagent I before using, and unused liquid can be stored at 2-8℃ for 4 weeks.
2. **Reagent II C:** Dissolve with 4 mL of Reagent I before using.It can be stored at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing.
3. **Reagent II D:** Dissolve with 2 mL of distilled water before using.It can be stored at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing.
4. **Preparation of Reagent II:** Before use, the Reagent II was prepared according to the sample size in the ratio of Reagent II A: Reagent II B: Reagent II C : Reagent II D = 100μL：500μL：150μL：150μL（5T）.
5. **Reagent III:** Dissolve with 0.5 mL of Reagent I one of the bottle before using, and unused liquid can be stored at 2-8℃ for 4 weeks.

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:

Ultraviolet Spectrophotometer/microplate reader, water-bath, table centrifuge, adjustable pipette, micro quartz cuvette/96 well UV plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. **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.
2. **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.
3. **Serum (plasma) sample:** direct detection. If there is precipitation, please centrifuge and take supernatant to be measured.

II. Determination procedure:

1. Preheat Ultraviolet spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 340 nm and set spectrophotometer counter to zero with distilled water.
2. Preheat Reagent II at 25℃ (other species) or 37℃ (mammals) water bath above 10 minutes.
3. Operation table:

Reagent Name (μL)	Test Tube (T)
Reagent II	180
Reagent III	10
Sample	10

The above reagents were added into a microquartz colorimetric dish or 96-well UV plate in sequence, immediately and thoroughly mixed, and then the absorption value A1 at 340nm was measured for 20s. The reagents were quickly placed at 37℃ (mammals) or 25℃ (other species) for an accurate reaction of 5min (the temperature can be adjusted to 37℃ with the temperature control function of the enzyme marker). The absorption value A1 at 340 nm and A2 at 5min and 5min later were recorded. Calculation ΔA=A2-A1

III. Calculation of HK activity:

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. 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.
   $HK(U/mg\ prot)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 10^9]\div(V_S\times Cpr)\div T=643\times\Delta A\div Cpr$
3. 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 mass)＝[ΔA×V_{TV}÷(ε×d)×10^9]÷(W ×V_S÷V_{TS})÷T=643×ΔA÷W

4. 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=1.286×ΔA

V_{TV}: Total volume of the reaction system, $2×10^{-4}$ L;
ε: The molar extinction coefficient of NADPH,$6.22×10^3$ 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. 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

3. 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 mass)＝[ΔA×V_{TV}÷(ε×d)×10^9]÷(W ×V_S÷V_{TS})÷T=1071.7×ΔA÷W

4. 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 104 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. The activity of HK in different homogenates is different. Please perform 1-2 preliminary experiments before the formal test. If ΔA is > 0.5, it means that the tissue activity is too high, and the homogenate supernatant must be diluted with the extraction solution to an appropriate concentration or the reaction time must be shortened to 2 minutes to make ΔA < 0.5 to improve the detection sensitivity. Please note that the calculation formula should be modified accordingly.

Experimental example:

1. Weigh about 0.0508g rat liver, add 1mL extract, homogenize in ice bath, 8000g, centrifuge at 4℃ for 10min, take the supernatant and put it on ice to be measured. Then, according to the measurement procedure, A1=0.183, A2=0.428, ΔA=A2-A1=0.245 were measured with 96-well UV plate, and the hexokinase activity was calculated as follows:
   HK activity (U/g mass) =1071.7×ΔA÷W=5168.632U/g mass.

Recent Product Citations：

[1] Gao J, Wang Z, Guo Q, Tang H, Wang Z, Yang C, Fan H, Zhang W, Ren C, Liu J. Mitochondrion-targeted supramolecular "nano-boat" simultaneously inhibiting dual energy metabolism for tumor selective and synergistic chemo-radiotherapy. Theranostics. 2022 Jan 1;12(3):1286-1302. doi: 10.7150/thno.67543. PMID: 35154487; PMCID: PMC8771563.
[2] Li M, Li H, Zhu Q, Liu D, Li Z, Chen H, Luo J, Gong P, Ismail AM, Zhang Z. Knockout of the sugar transporter OsSTP15 enhances grain yield by improving tiller number due to increased sugar content in the shoot base of rice (Oryza sativa L.). New Phytol. 2024 Feb;241(3):1250-1265. doi: 10.1111/nph.19411. Epub 2023 Nov 27. PMID: 38009305.
[3] Feng H, Tan J, Wang Q, Zhou T, Li L, Sun D, Fan M, Cheng H, Shen W. 伪-hederin regulates glucose metabolism in intestinal epithelial cells by increasing SNX10 expression. Phytomedicine. 2023 Mar;111:154677. doi: 10.1016/j.phymed.2023.154677. Epub 2023 Jan 23. PMID: 36724620.
[4] Han S, He Z, Hu X, Li X, Zheng K, Huang Y, Xiao P, Xie Q, Ni J, Liu Q. Inhibiting NLRP3 Inflammasome Activation by CY-09 Helps to Restore Cerebral Glucose Metabolism in 3×Tg-AD Mice. Antioxidants (Basel). 2023 Mar 15;12(3):722. doi: 10.3390/antiox12030722. PMID: 36978970; PMCID: PMC10045645.

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.

[2] Galina A, da Silva WS. Hexokinase activity alters sugar-nucleotide formation in maize root homogenates[J]. Phytochemistry, 2000, 53(1): 29-37.

Related Products:

BC0540/BC0545	Pyruvate Kinase(PK) Activity Assay Kit
BC0530/BC0535	Phosphofructokinase(PFK) Activity Assay Kit
BC2190/BC2195	Phosphoenolpyruvate Carboxylase(PEPC) Activity Assay Kit