Ver.250601

Hexokinase (HK) Activity Assay Kit

BC10001-02(100 Tests/96 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

I. Product Description

Hexokinase (HK) 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.

II. Reagent Composition & Preparation

Reagent	Volume	Storage
Extract Solution	110mL×1	2-8℃
Reagent I	25mL×1	2-8℃
Reagent II A	3 mL×1	2-8℃
Reagent II B	Powder×1	2-8℃
Dissolve with 12mL of Reagent I before using, and unused liquid can be stored at 2-8℃ for 4 weeks		
Reagent II C	Powder×1	-20℃
Dissolve with 4mL of Reagent I before using. It can be stored at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing		
Reagent II D	Powder×1	-20℃
Dissolve with 2mL of distilled water before using. It can be stored at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing.		
Reagent III	Powder×2	-20℃
Dissolve with 0.5mL of Reagent I one of the bottle before using, and unused liquid can be stored at 2-8℃ for 4 weeks.		

Note:

1. 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）.

III. Required but Not Provided

Ultraviolet spectrophotometer/Microplate reader, table centrifuge, water bath/ constant temperature incubator, adjustable pipette, micro quratz cuvette/ /96 well UV plate, mortar/homogenizer/ cell ultrasonic crusher, ice and distilled water.

IV. Protocol

I. Sample preparation

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 1mL Extract solution to 5 million bacteria or cells). Ultrasonic to break bacteria or cells (20% or 200 W on ice bath, ultrasound for 3seconds, interval of 10 seconds, repeat 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.
2. Tissue : The tissues mass (g): Extract solution volume (mL) is 1:5~10 (it is suggested that add 1mL 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 and sample list

1. Preheat ultraviolet spectrophotometer for 30 minute, adjust wavelength to 340 nm, set 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 A_1 at 340nm was measured for 20seconds. The reagents were quickly placed at 37℃ (mammals) or 25℃ (other species) for an accurate reaction of 5minute (the temperature can be adjusted to 37℃ with the temperature control function of the enzyme marker). The absorption value A1 at 340 nm and A_2 at 5minutes and 5minutes later were recorded. Calculation $\Delta A=A_2-A_1$.

III. Calculation for 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) = $[\Delta A\times V_{TV}\div(\varepsilon\times d)\times10^9]\div V_S\div T=643\times\Delta A$
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) = $[\Delta A\times V_{TV}\div(\varepsilon\times d)\times10^9]\div(V_S\times Cpr)\div T=643\times\Delta A\div Cpr$
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) = $[\Delta A\times V_{TV}\div(\varepsilon\times d)\times10^9]\div(W\times V_S\div V_{TS})\div T=643\times\Delta A\div W$
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) = $[\Delta A\times V_{TV}\div(\varepsilon\times d)\times10^9]\div(500\times V_S\div V_{TS})\div T=1.286\times\Delta A$

   V_{TV}: Total volume of the reaction system, 2×10^{-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) = $[\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^{9}] \div V_S \div T = 1071.7 \times \Delta A$
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) = $[\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^{9}] \div (V_S \times Cpr) \div T = 1071.7 \times \Delta A \div Cpr$
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) = $[\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^{9}] \div (W \times V_S \div V_{TS}) \div T = 1071.7 \times \Delta A \div W$
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) = $[\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^{9}] \div (500 \times V_S \div V_{TS}) \div T = 2.143 \times \Delta A$

   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.

Notes

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.