Hexokinase (HK) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC0740

Size: 50T/48S

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 60 mL×1	2-8℃
Reagent I	Liquid 30 mL×1	2-8℃
Reagent II	Powder×1	2-8℃
Reagent III	Liquid 5 mL×1	2-8℃
Reagent IV	Powder×1	-20℃
Reagent V	Powder×1	-20℃
Reagent VI	Powder×2	-20℃

Solution Preparation:

1. **Reagent II:** Dissolve with 30 mL of distilled water before using, and unused liquid can be stored at 2-8℃ for 4 weeks.
2. **Reagent IV :** Dissolve with 5 mL of distilled water before using.It can be stored at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing.
3. **Reagent V:** Dissolve with 3 mL of distilled water before using.It can be stored at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing.
4. **Reagent VI:** Before use, dissolve one reagent 6 by adding 125 μL of Reagent I and 125 μL of distilled water thoroughly, and keep it ready for use, and unused liquid can be stored at 2-8℃ for 2 weeks. (Note that this reagent is freeze-dried, and there may be noticeable variations in the apparent amount of reagent between vials, or even very small amounts visible. This phenomenon does not affect its use, as the actual quality remains the same.)

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, water-bath, table centrifuge, adjustable pipette, 1 mL quartz cuvette, 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 centrifugal tubes, 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 of Extract solution to 5 million bacteria or cells). Ultrasonic to broke break bacteria or cells (20% or 200W on ice bath, ultrasonic 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 of 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. Measurement operation:

1. Preheat spectrophotometer for more than 30 minutes, adjust the wavelength to 340 nm and set spectrophotometer counter to zero with distilled water.
2. Preheat reagent I, II, III, IV, V at 25℃ (other species) or 37℃ (mammals) water bath above 10 minutes.
3. Sample list:

Reagent Name (μL)	Test Tube
Reagent I	400
Reagent II	400
Reagent III	80
Reagent IV	80
Reagent V	40
Reagent VI	8
Sample	30

Add above reagents to a 1 mL quartz cuvette in sequence, mix immediately. Start timing at the same time as adding the sample, record the initial absorbance A1 at 20s under the 340 nm wavelength. Rapidly put cuvette and the reaction solution in 37℃(mammals) or 25℃ (other species) water-bath or incubator after colorimetric, react accurately for 5 minutes. Quickly take the cuvette and wipe dry it, colorimetric at 340 nm and record the absorbance A2 at 320 seconds, calculate ΔA = A2 - A1.

III. Calculation of HK activity:

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).

   $HK(U/mL)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 10^9]\div V_S\div T=1113\times\Delta A$

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) = [ΔA×V_{TV}÷(ε×d)×10^9]÷(V_S×Cpr) ÷T=1113×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=1113×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]÷(N×V_S÷V_{TS})÷T=1113×ΔA÷N

   V_{TV}: Total volume of the reaction system, 1.038×10^{-3} L;
   E: 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, 0.03 mL;
   V_{TS}: Add extract solution volume, 1 mL;
   T: Reaction time, 5 minutes;
   Cpr: Sample protein concentration, mg/mL;
   W: Sample mass, g;
   N: Total number of bacteria or cells, counted in tens of thousands.

Note:

1. The reaction solution in the 1 mL quartz 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 0.1g rat kidney, add 1mL extract, homogenize in ice bath, 8000g, centrifuge at 4℃ for 10min. Take the supernatant, dilute it 5 times with extraction solution, and then place it on ice for measurement. Then, follow the measurement procedures and use a 1mL quartz cuvette for the measurement and calculation, A1=0.2252, A2=0.3084, ΔA=A2-A1=0.0832, and the hexokinase activity was calculated as follows:

HK activity (U/g mass) =1113×ΔA÷W×dilution ratio =4630.08U/g mass.

2. Weigh about 0.1g Chlorophytum comosum, add 1mL extract, homogenize in ice bath, 8000g, centrifuge at 4℃ for 10min, and then place it on ice for measurement. Then, follow the measurement procedures and use a 1mL quartz cuvette for the measurement and calculation, A1=0.1949, A2=0.2184, ΔA=A2-A1=0.0235, and the hexokinase activity was calculated as follows:
HK activity (U/g mass) =1113×ΔA÷W =261.56U/g mass.

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:

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

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