Hexokinase (HK) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0809

Size: 50T/48S

Components:

Extract solution: 60 mL ×1, Storage at 4 ℃.

Reagent I: 30 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃. Add 30 mL of distilled water to dissolve fully before use. The unused reagent store at 4℃ for one week.

Reagent III: 5 mL×1. Storage at 4℃.

Reagent IV: Powder×1. Storage at -20℃. Add 4 mL of distilled water to dissolve fully for use, the unused reagent store at 4℃ for one week.

Reagent V: Powder×1. Storage at -20℃. Add 2 mL of distilled water to dissolve fully for use, the unused reagent store at 4℃ for one week.

Reagent VI: Powder×2. Storage at -20℃. Add 125 μL of Reagent I and 125 μL of distilled water to dissolve fully for use, the unused reagent store 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, water bath, centrifuge, adjustable pipette, 1 mL quartz cuvette, ice, mortar/ homogenizer and distilled water.

Procedure:

I. Sample preparation:

a.   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.

b. 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.

c. Serum (plasma) sample: direct detection.

II. Measurement operation:

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

b. Preheat reagent I, II, III, IV, V at 25℃ (other species) or 37℃ (mammals) water bath above 10 minutes.

c. 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 $\Delta A = A2 - A1$.

III. Calculation of HK activity calculation:

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. Calculation of HK activity in tissues, bacteria or cells:

a. 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.

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

b. 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.

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

c. 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.

$$HK(U/10^4 \text{ cell}) = [\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9] \div (500 \times V_S \div V_{TS}) \div T = 2.226 \times \Delta A$$

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;

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

Note:

1. If determine a large number of samples at one time, the Reagent I, II, III, IV, V and VI can be mixed in proportion to mixture solution and preheat for 10 minutes.

2. 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.

3. 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.

4. If $\Delta A > 0.5$, the tissue vitality is too high. In order to improve the detection sensitivity and make $\Delta A < 0.5$, 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：

NA0826/NA0584   Pyruvate Kinase(PK) Activity Assay Kit

NA0827/NA0585   Phosphofructokinase(PFK) Activity Assay Kit

NA0715/NA0474   Phosphoenolpyruvate Carboxylase(PEPC) Activity Assay Kit