Glucose-6-Phosphatase（G6P）Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: NA0392

Size:100T/48S

Components:

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

Reagent I: Liquid 12 mL×1. Storage at 4℃.

Reagent II: Powder×2. Storage at 4℃.

Reagent III: Powder×1. Storage at 4℃. Dissolve with 4 mL of distilled water before use.

Reagent IV: Powder×1. Storage at 4℃. Dissolve with 4 mL of distilled water before use.

Reagent V: Liquid 4 mL×1. Storage at 4℃.

Standard solution: 1 mL×1, 10 μmol/mL phosphorus standard solution.

Product Description:

Glucose-6-phosphatase (G6Pase, EC 3.1.3.9) is a kind of phosphatase which hydrolyzes phosphate compounds. It widely exists in animals, plants, microorganisms and cells. It is a restriction enzyme which hydrolyzes glucose-6-phosphate to produce glucose in the process of gluconeogenesis. It plays an important role in maintaining the dynamic balance of blood glucose.

G6P catalyzes glucose-6-phosphate to produce glucose and inorganic phosphorus. The increase of inorganic phosphorus content by molybdenum blue method can reflect the activity of G6P.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, low temperature desktop centrifuge, water bath pot, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/homogenizer, EP tube, ice and distilled water.

Procedure:

I. Extraction of crude enzyme solution:

1. Bacteria/cultured cells:

Collect bacteria/cells into the centrifuge tube first, and discard the supernatant after centrifugation. According to the number of bacteria/cells (10^4): the volume of the extract (mL) is 500-1000:1 (it is recommended to add 1 mL of the extract to 5 million bacteria/cells), ultrasonic wave breaks bacteria or cells (ice bath, power 20% or 200W, ultrasonic 3s, interval 10s, repeat 30 times). Centrifugate at 8000 g for 10 min at 4℃, take the supernatant and place it on ice for testing.

2. Tissue:

According to the proportion of tissue mass (g): extraction volume (mL) of 1:5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of extraction solution), carry out ice bath homogenization.

Centrifugate at 8000 g for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

3. Serum sample:

Direct detection.

II. Determination procedure:

1) Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 660 nm, set zero with distilled water.
2) Dilute 10 μmol/mL standard solution with distilled water 16 times to 0.625 μmol/mL standard solution for standby.
3) Preparation of working solution: add 5 mL of Reagent I into reagent II to fully dissolve.
4) Prepare of determining phosphorus reagent: make solution as the volume ratio of distilled water: Reagent III: Reagent IV: Reagent V=2:1:1:1. The prepared reagent shall be light yellow, if colorless means the reagent is fail, if blue means phosphorus pollution. Prepare the reagent when it will be use.
5) Operation table：

Reagent name (μL)	Test tube (A_T)	Contrast tube (Ac)	Standard tube (A_S)	Blank tube (A_B)
Sample	20	20		
Working solution	80	-		
Mix well and react in water bath at 37℃(mammal) or 25℃ (other species) for 10 minutes. After reaction, put it into boiling water for 10 minutes. Take out and cool to room temperature.				
Working solution	-	80		
Centrifugate at 10000 rpm for 10 minutes at normal temperature, then take the supernatant.				
Supernatant	25	25	-	-
Standard	-	-	25	-
determining phosphorus reagent	125	125	125	125
Distilled water	100	100	100	125
Mix well and react at 40℃ for 10 minutes. Suck 200 μL into a micro glass cuvette/96 well plate, measure the absorbance at 660 nm, and record the absorbance measured by the Test tube, the Contrast tube, the Blank tube and the Standard tube as A_T, A_C, A_B and A_S respectively. Calculate $\Delta A = A_T - A_c$, $\Delta A_S = A_S - A_B$.				

III. Calculation of G6P:

1. Calculation of serum (plasma) G6P activity

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of generates 1 nmol of inorganic phosphorus per minute every milliliter of serum (plasma).

G6P (U/mL)=$\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_{EM} \div V_S \div T = 312.5 \times \Delta A \div \Delta A_S$.

2. Calculation of G6P activity in tissues, bacteria or cells

(1) Calculated by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the

generation of generates 1 nmol of inorganic phosphorus per minute every milligram of tissue protein.

G6P (U/mg prot) =$\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_{EM} \div (Cpr \times V_S) \div T = 312.5 \times \Delta A \div \Delta A_S \div Cpr$.

(2) Calculated by fresh weight of sample

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of generates 1 nmol of inorganic phosphorus per minute every per gram of tissue weight.

G6P (U/g fresh weight) =$\Delta A \div (\Delta A_S \div Cs) \times 1000 \times V_{EM} \div (W \div V_E \times V_S) \div T = 312.5 \times \Delta A \div \Delta A_S \div W$.

(3) According to the density of bacteria or cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of generates 1 nmol of inorganic phosphorus per minute every 10 thousand bacteria or cells.

G6P (U/10^4 cell) =$\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_{EM} \div (500 \div V_E \times V_S) \div T = 0.625 \times \Delta A \div \Delta A_S$.

C_S: Concentration of standard solution, 0.625 μmol/mL;

V_{EM}: Total volume of enzymatic reaction, 0.1 mL;

V_S: Sample volume, 0.02 mL;

V_E: Sample volume, 1 mL;

T: Reaction time, 10 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

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

1000: Unit conversion coefficient, 1 μmol=1000 nmol.

Note:

1. It is recommended that the sample be diluted with the extract before determination, and multiplied by the dilution ratio in the calculation formula.
2. If A is greater than 1.5 or there is precipitation after color development, dilute the supernatant or crude enzyme solution with distilled water before determination.
3. Phosphorus determination reagent should be prepared when the solution will be used, the normal color is light yellow, if there is discoloration or blue, it will be invalid.

Experimental examples:

1. Take 0.1 g of mouse liver tissue and add 1 mL of Extract solution for sample processing. After centrifugation to take the supernatant, proceed according to the determination procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A = A_T - A_C = 0.995 - 0.384 = 0.611$, $\Delta A_S = A_S - A_B = 0.357 - 0.047 = 0.31$. The enzyme activity is calculated according to the sample mass.
   G6P (U/g fresh weight) =$312.5 \times \Delta A \div \Delta As \div W$=6159.274 U/g fresh weight.
2. Take 0.1 g of barnyardgrass and add 1 mL of Extract solution for sample processing. After centrifugation to take the supernatant, proceed according to the determination procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A = A_T - A_C = 0.995 - 0.384 = 0.611$, $\Delta A_S = A_S - A_B = 0.357 - 0.047 = 0.31$. The enzyme activity is calculated according to the sample mass.
   G6P (U/g fresh weight) =$312.5 \times \Delta A \div \Delta As \div W$=896.4646 U/g fresh weight.
3. The mouse serum was diluted 2 times and tested directly. After determination with 96 well flat-bottom

plate, calculate $\Delta A=A_T-A_C=0.995-0.384=0.611$，$\Delta A_S=A_S-A_B=0.357-0.047=0.31$. The enzyme activity is calculated according to the serum volume.

G6P (U/mL)=$312.5\times\Delta A\div\Delta A_S\times2$(dilution times)=364.9194 U/mL.

Recent Product citations:

[1] Fan X, Hou T, Jia J, et al. Discrepant dose responses of bisphenol A on oxidative stress and DNA methylation in grass carp ovary cells[J]. Chemosphere, 2020, 248: 126110.

Related products:

NA0810/NA0568 Pyruvate Carboxylase(PC) Activity Assay Kit

NA0801/NA0560 Fructose 1,6-bisphosphatase(FBP) Activity Assay Kit