



E-mail:sales@sunlongbiotech.com

www.sunlongbiotech.com

# Glucose-6-Phosphatase (G6P) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Cat No:** AK0141 **Size:**50T/24S

# **Components:**

Extract solution: Liquid 40 mL×1. Storage at 4°C.

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

Reagent II: Powder×2. Storage at 4°C.

Reagent III: Powder×1. Storage at 4°C. Dissolve with 8 mL of distilled water before use.

Reagent IV: Powder×1. Storage at 4°C. Dissolve with 8 mL of distilled water before use.

Reagent V: Liquid 8 mL×1. Storage at 4°C.

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:

Visible spectrophotometer, low temperature desktop centrifuge, water bath pot, 1 mL glass cuvette, 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, discard the supernatant after centrifugation. According to the number of bacteria/cells (10<sup>4</sup>): 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 minutes at 4°C, take the supernatant and place it on ice for testing.

#### 2. Tissue:

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

E-mail:sales@sunlongbiotech.com

Tel: 0086-571-56623320 Fax:0086-571-56623318

www.sunlongbiotech.com

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

3. Serum sample:

Direct detection.

# **II. Determination procedure:**

- 1) Preheat spectrophotometer for 30 minutes, adjust the wavelength to 660 nm, set zero with distilled
- 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 for standby. The working solution can be stored at -20°C after sub loading, and repeated freeze-thaw is prohibited.
- 4) Preparation of determining phosphorus reagent: make solution as the volume ratio of H2O: 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 used.

#### Operation table: 5)

Reagent name (μL)	Test tube	Contrast tube (Ac)	Standard	Blank
	$(A_T)$		tube $(A_S)$	tube
				$(A_B)$
Sample	40	40		
Working solution	160			
Mix well and react in water bath at 37°C(mammal) or 25°C (other				
species) for 10 minutes. After reaction, put it into boiling water for				
10 minutes. Take out and cool to room temperature.				
Working solution	_	160		
Centrifugate at 10000 rpm for 10 minutes at normal temperature,				
then take the supernatant.				
Supernatant	100	100	_	_
Standard	_	_	100	_
Determining phosphorus reagent	500	500	500	500
Distilled water	400	400	400	500

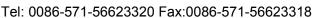
Mix well and react at 40°C for 10 minutes. Measure the absorbance at 660 nm, and record the absorbance measured by the Test tube, the Contrast tube, the Blank tube and the Standard tube as A<sub>T</sub>, A<sub>C</sub>, A<sub>B</sub> and  $A_S$  respectively. Calculate  $\Delta A = A_T - A_C$ ,  $\Delta A_S = A_S - A_B$ .

# 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







www.sunlongbiotech.com

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) = 
$$\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_{EM} \div (W \div V_E \times V_S) \div T = 312.5 \times \Delta A \div \Delta A_S \div W$$
.

(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.

$$G6P\left(U/10^{4}\,cell\right) = \Delta A \div (\Delta A_{S} \div C_{S}) \times 1000 \times V_{EM} \div (500 \div V_{E} \times V_{S}) \div T = 0.625 \times \Delta A \div \Delta A_{S} \; .$$

C<sub>S</sub>: Concentration of standard solution, 0.625 μmol/mL;

V<sub>EM</sub>: Total volume of enzymatic reaction, 0.2 mL;

V<sub>S</sub>: Sample volume, 0.04 mL;

V<sub>E</sub>: Sample volume, 1 mL;

T: Reaction time, 10 min;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

500: Total number of bacteria or cells, 500 ×10 thousand;

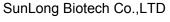
1000: Unit conversion coefficient, 1 μmol=1000 nmol.

#### 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 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 muscle tissue and add 1 mL of Extract solution for sample processing. After centrifugation to take the supernatant, proceed according to the determination procedure. Calculate  $\Delta A = A_T A_C = 0.913 0.869 = 0.044$ ,  $\Delta A_S = A_S A_B = 0.509 0.014 = 0.495$ . The enzyme activity is calculated according to the sample mass.
  - G6P (U/g fresh weight) =  $312.5 \times \Delta A \div \Delta As \div W = 277.7778$  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. Calculate  $\Delta A = A_T A_C = 0.379 0.237 = 0.142$ ,  $\Delta A_S = A_S A_B = 0.509 0.014 = 0.495$ . 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.





Tel: 0086-571-56623320 Fax:0086-571-56623318

E-mail:sales@sunlongbiotech.com

www.sunlongbiotech.com

# **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:**

AK0317/AK0316 Pyruvate Carboxylase(PC) Activity Assay Kit

AK0276/AK0275 Fructose 1,6-bisphosphatase(FBP) Activity Assay Kit