

6-Phosphate Dehydrogenase (G6PDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0570

Size:50T/48S

Components:

Extract solution: Liquid 50 mL×1. Storage at 4°C .

Reagent I: Liquid 50 mL×1. Storage at 4°C . **Avoid placing at -20°C.**

Reagent II: Powder×1. Storage at -20°C . Dissolve with 650 μL of distilled water before use.

Reagent III: Powder×1. Storage at -20°C . Dissolve with 650 μL of distilled water before use.

Product Description:

6-Phosphate dehydrogenase (G6PDH) is widely found in animals, plants, microorganisms and cultured cells. It is a key enzyme in the pentose phosphate pathway, which catalyzes the oxidation of glucose 6-phosphate to glucose 6-phosphate lactone. In the meantime, G6PDH reduces NADP⁺ to NADPH, which provides biosynthesis and maintain the reduction state of cells. The activity of G6PDH can reflect the biosynthesis level and antioxidant capacity of the organisms.

G6PDH catalyzes the conversion of NADP⁺ to NADPH. In this kit, the activity of G6PDH is determined by the increase rate of NADPH at 340 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, refrigerated centrifuge, adjustable transferpettor, water bath, 1 mL quartz cuvette, mortar/ homogenizer, ice and distilled water.

Procedure:

I. Sample preparation:

1. Bacteria or cells

Collecting bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation. It is recommended to add 1 mL of Extract reagent to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 20%, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice for testing.

2. Tissue

Add 1 mL of Extract reagent to 0.1 g of tissue, fully homogenized on ice. Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice for testing.

3. Serum (plasma) sample:

Detect sample directly.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, set to zero with distilled water.
2. Preheat Reagent I for 30 minutes at 37°C .
3. Working solution: Reagent I, Reagent II and Reagent III are mixed by the ratio of 15 : 0.2 : 0.2.
4. Add the following reagents

Reagent (μL)	Test tube (T)	Blank tube (B)
Working solution	950	950
Sample	50	-
Distilled water	-	50

Detect the absorbance of initial and final reaction at 340 nm, record as A1(0s), A2(5min).
 $\Delta A(\text{Test}) = \Delta A(T) = A2(T) - A1(T)$, $\Delta A(\text{Blank}) = \Delta A(B) = A2(B) - A1(B)$.

III. Calculation:

1. Serum (plasma) sample

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADPH in the reaction system per minute every milliliter serum (plasma).

$$\text{G6PDH (U/mL)} = [(\Delta A(T) - \Delta A(B)) \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div V_s \div T = 643 \times [\Delta A(T) - \Delta A(B)]$$

2. Tissue

- 1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADPH in the reaction system per minute every milligram protein.

$$\text{G6PDH (U/mg prot)} = [(\Delta A(T) - \Delta A(B)) \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (V_s \times C_{pr}) \div T = 643 \times [\Delta A(T) - \Delta A(B)] \div C_{pr}$$

- 2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADPH in the reaction system per minute every gram tissue.

$$\text{G6PDH (U/g weight)} = [(\Delta A(T) - \Delta A(B)) \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (W \div V_e \times V_s) \div T = 643 \times [\Delta A(T) - \Delta A(B)] \div W$$

3. Bacteria or cultured cells

- 1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADPH in the reaction system per minute every milligram protein.

$$\text{G6PDH (U/mg prot)} = [(\Delta A(T) - \Delta A(B)) \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (V_s \times C_{pr}) \div T = 643 \times [\Delta A(T) - \Delta A(B)] \div C_{pr}$$

- 2) Amount of cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADPH in the reaction system per minute every 10⁴ cells or bacteria.

$$\text{G6PDH (U/10}^4 \text{ cell)} = [(\Delta A(T) - \Delta A(B)) \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (V_s \div V_e \times 500) \div T = 1286 \times [\Delta A(T) - \Delta A(B)]$$

ϵ : NADPH molar extinction coefficient, 6.22×10³ L/mol/cm;

d: Light path of cuvette, 1 cm;

V_{rv}: Total reaction volume, 0.001 L;

Vs: Supernate volume, 0.05 mL;
Ve: Extract volume, 1 mL;
Cpr: Sample protein concentration, mg/mL;
T: Reaction time, 5 minutes;
W: Sample weight, g;
500: 5 million cells or bacteria;
 10^9 : Unit conversion factor, 1 mol = 10^9 nmol.

Note:

1. During the experiment, keep samples on ice to avoid denaturing and inactivating and place Reagent I in 37°C waterbath to keep warm.
2. During the experiment, reaction solution must be keep a constant temperature of about 37°C . Take a small beaker and put a certain amount of 37°C distilled water into it. Put the beaker into a 37°C water bath pot. Put the cuvette and reaction solution into the beaker during the reaction.
3. Suggest two people cooperate in this experiment (one for adding samples, one for record).
4. If $\Delta A < 0.1$ and $A_1(0s) > 0.5$, the sample can be determined after being appropriately diluted.

Experimental example:

About 0.1 g of spleen is weighed and 1 mL of Extract solution is added for ice bath homogenization. After centrifugation at 4°C for 10 min, the supernatant is taken out, and then the operation is carried out according to the determination steps. The results showed that $\Delta A_T = A_{T2} - A_{T1} = 1.234 - 0.479 = 0.755$, $\Delta A_B = A_{B2} - A_{B1} = 0.034 - 0.022 = 0.012$

$G6PDH (U/g \text{ mass}) = 643 \times (\Delta A_T - \Delta A_B) \div W = 643 \times 0.734 \div 0.1 = 4777.49 U/g \text{ mass}$.

Recent Product Citations:

[1] Yang Y, Liu W, Li D, et al. Altered glycometabolism in zebrafish exposed to thifluzamide[J]. Chemosphere, 2017, 183: 89-96.

[2] Wu S, Wang H, Li Y, et al. Transcription factor YY1 promotes cell proliferation by directly activating the pentose phosphate pathway[J]. Cancer research, 2018, 78(16): 4549-4562.

[3] 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.

References:

[1] Anderson B M, Wise D J, Anderson C D. Azotobacter vinelandii glucose 6-phosphate dehydrogenase properties of NAD- and NADP-linked reactions[J]. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology, 1997, 1340(2): 268-276.

Related Products:

AK0488/AK0487 NADP Phosphatase(NADPase) Activity Assay Kit
AK0550/AK0549 Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Activity Assay Kit
AK0486/AK0485 NADP Malic Enzyme(NADP-ME) Activity Assay Kit
AK0408/AK0407 6PGDH Activity Assay Kit