6-Phosphate Dehydrogenase (G6PDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC0260

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 50 mL×1	2-8℃
Reagent I	Liquid 50 mL×1	2-8℃ (Do not put -20℃)
Reagent II	Powder×1	2-8℃
Reagent III	Powder×1	2-8℃

Solution Preparation:

1. Reagent II: When used, add 650μL distilled water to each branch and fully dissolve it for use.

2. Reagent III: When used, add 650μL distilled water to each branch and fully dissolve it for use.

3. Preparation of working liquid: Before use, the working liquid was prepared according to the sample size in the ratio of Reagent I: Reagent II: Reagent III =15: 0.2: 0.2.

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.

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Reagents and Equipment Required but Not Provided:

ultraviolet spectrophotometer, refrigerated centrifuge, adjustable pipette, water bath, 1 mL quartz cuvette, mortar/ homogenizer, 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 cells: The ratio of bacteria/cell amount (10^4): the volume of Extract solution (mL) is

500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s，interval 10 s，repeat 30 times). Centrifuge at 8000g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

2. Tissue sample: It is recommended to weigh 0.1g sample and add 1.0mL Extract solution, after ice bath homogenization, centrifuge at 4℃, 8000g for 10min, take supernatant and placed on the ice for test.

3. Serum (plasma) sample: Detect sample directly.

II. Determination procedure:

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

2. Preparation of working liquid for 10 minutes at 37℃.

3. Add the following reagents

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

Mix well in a micro quartz cuvette or a 96 well UV plate, and start timing while adding the sample. Detect the absorbance at 340 nm at the time of 0 seconds record as A1. After colorimetry, quickly place the micro quartz cuvette together with the reaction solution into a 37 ℃ water bath or constant temperature incubator to accurately react for 5 minutes. (If the microplate reader has temperature control function, adjust the temperature to 37℃). Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2(5 min). $\Delta A_T=A2_T-A1_T$, $\Delta A_B=A2_B-A1_B$. (The blank tube only need to be measured 1-2 times.)

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).

G6PDH (U/mL) =$[(\Delta A_T-\Delta A_B) \div(\varepsilon\times d) \times10^9\times Vrv] \div Vs \div T=643\times(\Delta A_T-\Delta A_B)$

2. Tissue sample:

1) Calculate by sample 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.

G6PDH (U/mg prot) =$[(\Delta A_T-\Delta A_B) \div(\varepsilon\times d) \times10^9\times Vrv] \div(Vs\times Cpr) \div T=643\times(\Delta A_T-\Delta A_B) \div Cpr$

2) Calculate by sample mass:

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.

G6PDH (U/g mass) =$[(\Delta A_T-\Delta A_B) \div(\varepsilon\times d) \times10^9\times Vrv] \div(W\div Ve\times Vs) \div T=643\times(\Delta A_T-\Delta A_B) \div W$

3. Bacteria or cultured cells

1) Calculate by sample 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.

G6PDH (U/mg prot)=[(ΔA_T-ΔA_B)÷(ε×d)×10^9×Vrv]÷(Vs×Cpr)÷T=643×(ΔA_T-ΔA_B)÷Cpr

2) Calculate by the number of bacteria or cells:

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 10^4 cells or bacteria.

G6PDH(U/10^4 cell)=[(ΔA_T-ΔA_B)÷(ε×d)×10^9×Vrv]÷(Vs÷Ve×500)÷T=1.286×(ΔA_T-ΔA_B)

ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: Light path of cuvette, 1 cm;

Vrv: 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 denaturating and inactivating and place Reagent I in 37℃ waterbath to keep warm.
2. During the experiment, reaction solution must be keep a constant temperature of about 37℃. Take a small beaker and put a certain amount of 37℃ distilled water into it. Put the beaker into a 37℃ 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 ΔA<0.1 and A1(0s)>0.5, the sample can be determined after being appropriately diluted.

Experimental example:

1. About 0.1g of spleen is weighed and 1 mL of Extract solution is added for ice bath homogenization. After centrifugation at 4℃ for 10 min, the supernatant is taken out, and then the operation is carried out according to the determination steps. The results showed that ΔA_T = $A2_T$ – $A1_T$ = 1.234-0.479 = 0.755, ΔA_B= $A2_B$ – $A1_B$= 0.034-0.022 = 0.012

G6PDH (U/g mass) = 643 × (ΔA_T- ΔA_B) ÷ W = 643 × 0.734 ÷ 0.1 = 4777.49 U/g 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.

[4] Ran Y, Yang Q, Zeng J, Li F, Cao Y, Xu Q, Qiao D, Xu H, Cao Y. Potential xylose transporters regulated by CreA improved lipid yield and furfural tolerance in oleaginous yeast Saitozyma podzolica zwy-2-3. Bioresour Technol. 2023 Oct;386:129413. doi: 10.1016/j.biortech.2023.129413. Epub 2023 Jun 28. PMID: 37390935.

[5] Wu D, Guo J, Zhang Q, Shi S, Guan W, Zhou C, Chen R, Du B, Zhu L, He G. Necessity of rice resistance to planthoppers for OsEXO70H3 regulating SAMSL excretion and lignin deposition in cell walls. New Phytol. 2022 May;234(3):1031-1046. doi: 10.1111/nph.18012. Epub 2022 Feb 26. PMID: 35119102; PMCID: PMC9306520.

References:

[1] Rüdiger Hauschild, Antje von Schaewen. Differential Regulation of Glucose-6-Phosphate Dehydrogenase Isoenzyme Activities in Potato [J]. Plant Physiology, 2003, 133(1): 47-62.

[2] Philip E, Pamela J, David I. et al. Methylglyoxal-induced modification of arginine residues decreases the activity of NADPH-generating enzymes [J]. Free Radical Biology and Medicine, 2013, 61: 229-242.

[3] Rüdiger Hauschild, Antje von Schaewen. Differential Regulation of Glucose-6-Phosphate Dehydrogenase Isoenzyme Activities in Potato [J]. Plant Physiology, 2003, 133(1): 47-62.

[4] Philip E, Pamela J, David I. et al. Methylglyoxal-induced modification of arginine residues decreases the activity of NADPH-generating enzymes [J]. Free Radical Biology and Medicine, 2013, 61: 229-242.

[5] 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.

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BC0400/BC0405	Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Activity Assay Kit
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BC2100/BC2105	6PGDH Activity Assay Kit

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