6-Phosphate Dehydrogenase(G6PDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: BC0265

Size:100T/96S

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 100 mL×1	2-8℃
Reagent I	Liquid 20 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 250μL distilled water to each branch and fully dissolve it for use.

2. Reagent III: When used, add 250μL 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.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, micro quartz cuvette/96 well UV flat-bottom plate, water bath, refrigerated centrifuge, adjustable transferpettor, mortar/ homogenizer, ice and distilled water.

Procedure:

I. Sample preparation:

1. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. 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, 200W, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ 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, and fully homogenized on ice. Centrifuge at 8000 ×g for 10 minutes at 4℃ 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/ microplate reader for 30 minutes, adjust the wavelength to 340 nm, set the counter to zero with distilled water.
2. Preheat Reagent I for 30 minutes at 37℃.
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	190	190
Sample	10	-
Distilled H_2O	-	10

Detect the absorbance of initial and final reaction at 340 nm, record as A1(0s), A2(5min). ΔA(Test)=ΔA(T)=A2(T)-A1(T), ΔA(Blank)=ΔA(B)=A2(B)-A1(B).

III. Calculation:

a. micro quartz cuvette

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)=[(ΔA(T)-ΔA(B))÷(ε×d)×10^9×Vrv]÷Vs÷T=643×[ΔA(T)-ΔA(B)]

2. 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

3. 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.

G6PDH (U/g weight)=[(ΔA(T)-ΔA(B))÷(ε×d)×10^9×Vrv]÷(W÷Ve×Vs)÷T=643×[ΔA(T)-ΔA(B)]÷W

4. 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.

G6PDH (U/10^4 cell)=[(ΔA(T)-ΔA(B))÷(ε×d)×10^9×Vrv]÷(Vs÷Ve×500)÷T=1286×[ΔA(T)-ΔA(B)].

ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of micro quartz cuvette, 1 cm;
Vrv: Total reaction volume, 2×10^{-4} L;
Vs: Supernate volume (mL), 0.01 mL;
Ve: Extract volume, 1 mL;
Cpr: Sample protein concentration, mg/mL;
T: Reaction time (min), 5 minutes;

W: Sample weight, g;
500: 5 million cells;
10^9: Unit conversion factor, 1 mol =10^9 nmol.

b. 96 well UV flat-bottom plate

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)=[ΔA(T)-ΔA(B)]÷(ε×d)×10^9×Vrv÷Vs÷T=1072×[ΔA(T)-ΔA(B)]

2. 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=1072×[ΔA(T)-ΔA(B)]÷Cpr

3. 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.

G6PDH (U/g weight)=[(ΔA(T)-ΔA(B))÷(ε×d)×10^9×Vrv]÷(W÷Ve×Vs)÷T=1072×[ΔA(T)-ΔA(B)]÷W

4. 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.

G6PDH (U/10^4 cell)=[(ΔA(T)-ΔA(B))÷(ε×d)×10^9×Vrv]÷(Vs÷Ve×500)÷T=2.144×[ΔA(T)-ΔA(B)]

ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of cuvette, 0.6 cm;
Vrv: Total reaction volume, 2×10^{-4} L;
Vs: Supernate volume (mL), 0.01 mL;
Ve: Extract volume, 1 mL;
Cpr: Sample protein concentration, mg/mL;
T: Reaction time (min), 5 minutes;
W: Sample weight, g;
500: 5 million cells;
10^9: Unit conversion factor, 1 mol =10^9 nmol.

Note:

1. During the experiment, keep samples on ice to avoid denaturating and inactivating. Place Reagent I in 37℃ water bath 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.

5. In this kit , the alculate of enzyme activity is according to react rate, when using 96 well UV flat-bottom plate, do not detect excessive samples simultaneously to guarantee the same react time for every samples.

Experimental example:

1.About 0.1 g of spleen is weighed and 1 mL of Extract solution is added for ice bath homogenization. After centrifugation at 8000g 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 $\Delta A_T = A_{T2} - A_{T1} = 1.2906-0.4521=0.8385$, $\Delta A_B = A_{B2} - A_{B1} = 0.0276-0.026=0.0016$

G6PDH (U/g mass) = $643 \times (\Delta A_T - \Delta A_B) \div W = 643 \times 0.8369 \div 0.1 = 5381.267$ U/g mass.

2. The rabbit serum is directly measured according to the determination procedure. The enzyme activity is calculated according to the liquid volume: $\Delta A_T = A_{T2} - A_{T1} = 0.1528-0.136 = 0.0168$, $\Delta A_B = A_{B2} - A_{B1} = 0.0276-0.026 = 0.0016$

G6PDH (U/mL) = $643 \times (\Delta A_T - \Delta A_B) = 643 \times 0.0152 = 9.7736$ U/mL.

Recent Product Citations:

[1] Ma H, Zhang F, Zhou L, Cao T, Sun D, Wen S, Zhu J, Xiong Z, Tsau MT, Cheng ML, Hung LM, Zhou Y, Li Q. c-Src facilitates tumorigenesis by phosphorylating and activating G6PD. Oncogene. 2021 Apr;40(14):2567-2580. doi: 10.1038/s41388-021-01673-0. Epub 2021 Mar 8. PMID: 33686238.

[2] Tian Y, Li Y, Sun S, Dong Y, Tian Z, Zhan L, Wang X. Effects of urban particulate matter on the quality of erythrocytes. Chemosphere. 2023 Feb;313:137560. doi: 10.1016/j.chemosphere.2022.137560. Epub 2022 Dec 13. PMID: 36526140.

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.

Related Products:

BC1110/BC1115 NADP Phosphatase(NADPase) Activity Assay Kit
BC0400/BC0405 Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Activity Assay Kit
BC1120/BC1125 NADP Malic Enzyme(NADP-ME) Activity Assay Kit
BC2100/BC2105 6PGDH Activity Assay Kit