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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: AK0569

Size:100T/96S

Components:

Extract solution: Liquid 100 mL×1. Storage at 4℃ .

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

Reagent II: Powder×1. Storage at 4℃ . Dissolve with 250 μL of distilled water before use.

Reagent III: Powder×1. Storage at 4℃ . Dissolve with 250 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/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, ultrasonic power 20%, 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. 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.

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

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.

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

3. Cells or bacteria:

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.

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

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.

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. 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.

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

3. Cells or bacteria:

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.

G6PDH (U/mg prot)=[(ΔA(T)-ΔA(B))÷(ε×d)×10^9 ×Vrv]÷(Vs×Cpr)÷T=1072×[ΔA(T)-ΔA(B)]÷Cpr

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.

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 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 × (ΔA_T- ΔA_B) ÷ W = 643×0.8369÷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 × (ΔA_T - ΔA_B) = 643 × 0.0152 = 9.7736 U/mL.

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

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AK0550/AK0549	Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Activity Assay Kit
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