6-phosphogluconate Dehydrogenase(6-PGDH) Activity Assay Kit

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

Operation Equipment: Microplate Reader/Spectrophotometer

Cat No: NA0479

Size:100T/96S

Components:

Reagent I: 60 mL×2. Store at 4℃.

Reagent II: Powder×1. Store at -20℃. Dissolve with 2.2 mL of Reagent I before use.

Reagent III: Powder×1. Store at 4℃. Dissolve with 2 mL of Reagent I before use.

Product Description:

The 6-phosphate glucose dehydrogenase (6-PGDH) and 6-phosphogluconate dehydrogenase (6-PGDH) in the pentose phosphate pathway catalyze the synthesis of NADPH in turn, which is closely related to energy balance, growth rate and cell viability. In addition, 6-PGDH plays an important role in stress physiology. 6-PGDH catalyzes the production of NADPH by 6-phosphogluconic acid and $NADP^+$. NADPH has a characteristic absorption peak at 340 nm, while $NADP^+$ does not. In this kit, the activity of 6-PGDH is determined by the increase rate of NADPH at 340 nm.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, pipette, water bath, micro quartz cuvette/96 well UV plate, mortar/homogenizer, ice, distilled water.

Procedure:

I. Sample preparation:

Add 1 mL of Reagent I to 0.1 g of tissue and fully homogenized on ice bath. Centrifuge at 10000 rpm for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer or microplate reader for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.
2. Preheat Reagent I for 30 minutes at 37℃ water bath.
3. Add the following reagents

Reagent (μL)	Test tube (T)	Blank tube (B)
Sample	20	-
Distilled water	-	20
Reagent I	140	140
Reagent II	20	20
Reagent III	20	20

Mix thoroughly and timing, detect the absorbance of initial and final reaction at 340 nm, record as A1(0s) and A2(3 min) respectively. ΔA(Test)=ΔA(T)=A2(T)-A1(T), ΔA(Blank)=ΔA(B)=A2(B)-A1(B).

III. Calculation:

a. micro quartz cuvette

1) Protein concentration:

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

6-PGDH (U/mg prot)=[ΔA(T)-ΔA(B)]÷(ε×d)×10^6×Vrv÷(Vs×Cpr)÷T=536×[ΔA(T)-ΔA(B)]÷Cpr

2) Sample weight:

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

6-PGDH (U/g weight)=[ΔA(T)-ΔA(B)]÷(ε×d)×10^6×Vrv÷(W÷Ve×Vs)÷T=536×[ΔA(T)-ΔA(B)]÷W

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

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume, 0.0002 L;

Cpr: Sample protein concentration (mg/mL); Protein concentration needs to be determined additionally. BCA protein content determination kit of our company is recommended.

Vs: Supernatant volume (mL), 0.02 mL;

Ve: Volume of Reagent I added during extraction, 1 mL;

T: Reaction time (min), 3 minutes;

W: Sample weight(g);

b. 96 well UV plate

1）Protein concentration:

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

6-PGDH (U/mg prot)=[ΔA(T)-ΔA(B)]÷(ε×d)×10^6×Vrv÷(Vs×Cpr)÷T=893×[ΔA(T)-ΔA(B)]÷Cpr

2）Sample weight:

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

6-PGDH (U/g weight)=[ΔA(T)-ΔA(B)]÷(ε×d)×10^6×Vrv÷(W÷Ve×Vs)÷T=893×[ΔA(T)-ΔA(B)]÷W

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

d: Light path of cuvette, 0.5 cm;

Vrv: Total reaction volume, 0.0002 L;

Cpr: Sample protein concentration (mg/mL); Protein concentration needs to be determined additionally. BCA protein content determination kit of our company is recommended.

Vs: Supernatant volume (mL), 0.02 mL;

Ve: Volume of Reagent I added during extraction , 1 mL;

T: Reaction time (min), 3 minutes;
W: Sample weight(g);

Note:

1. Reagent II and Reagent III need be prepared when the solution will be used and the unused reagents can be stored at 4℃ for one week.
2. During the test, keep samples on ice to avoid denaturating and inactivating. The enzyme activity should be determined on the day of extract, and the crude enzyme solution should avoid repeated freezing and thawing.
3. If the initial (0s) reading value of the sample is greater than 0.5 and the determination of ΔA is less than 0.1, the sample can be diluted for determination.
4. When 96 well UV plate is used for determination, Reagent I (after preheating), Reagent II and Reagent III can be premixed into working solution according to the number of samples. Because the enzyme activity is calculated according to the reaction rate. In order to ensure that the reaction time of each sample is as consistent as possible, it is not recommended to measure too many samples at the same time.

Experimental example:

1. Weigh about 0.1g kidney tissue, add 1ml reagent 1, grind it on ice, centrifuge it at 10000rpm 4 ℃ for 10min, dilute the supernatant 5 times, measure it with micro quartz plate, calculate $\Delta A_T=A2_T - A1_T=0.4866-0.3035=0.1831$, $\Delta A_B=A2_B - A1_B=0.0325-0.0269=0.0056$, calculate the enzyme activity according to the sample mass.

6PGDH enzyme activity (U/g mass) = 536 × (ΔAT- ΔAB) × W × 5 (dilution ratio) = 4757 U/g mass.

Recent Product Citations:

[1] 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.

Related Products:

NA0787/NA0546 NADP Phosphatase(NADPase) Activity Assay Kit
NA0848/NA0606 G6PDH Activity Assay Kit
NA0835/NA0593 Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Activity Assay Kit
NA0786/NA0545 NADP Malic Enzyme(NADP-ME) Activity Assay Kit