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

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

Operation Equipment: Spectrophotometer

Cat No: BC2100

Size:50T/48S

Components:

Reagent I: 100 mL×1. Store at 4℃.

Reagent II: Powder×1. Store at -20℃. Dissolve with 5 mL of Reagent I before use.

Reagent III: Powder×1. Store at 4℃. Dissolve with 5 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, desk centrifuge, pipette, water bath, 1 mL quartz cuvette, 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 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	100	-
Distilled water	-	100
Reagent I	700	700
Reagent II	100	100
Reagent III	100	100

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:

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, 0.001 L;

Vs: Supernate volume (mL), 0.1 mL;

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

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

T: Reaction time (min), 3 minutes;

W: Sample weight(g);

Note:

1. 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.
2. 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.
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.

Experimental example:

1. Weigh about 0.1g of kidney tissue, add 1 mL of Reagent I, grind it on ice, centrifuge it at 10000 rpm and 4℃ for 10 min, take the supernatant and dilute it 10 times. Calculate $\Delta A_T = A2_T - A1_T$ = 0.256-0.154 = 0.102, $\Delta A_B = A2_B - A1_B$ = 0.

6-PGDH enzyme activity (U/g mass) = 536× ($\Delta A_T - \Delta A_B$) × W × 10 (dilution ratio) = 5467.2 U/g mass.

Related 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：

BC1110/BC1115 NADP Phosphatase(NADPase) Activity Assay Kit
BC0260/BC0265 G6PDH Activity Assay Kit
BC0400/BC0405 Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Activity Assay Kit
BC1120/BC1125 NADP Malic Enzyme(NADP-ME) Activity Assay Kit