Glycogen Phosphorylase a (GPa) Activity Assay Kit

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

Operation Equipment: Ultraviolet spectrophotometer/Microplate reader

Catalog Number: NA0390

Size: 100T/96S

Components:

Reagent	Size	Storage
Extract solution	110 mL×1	4℃
Reagent I	20 mL×1	4℃
Reagent II	Powder×1	4℃
Reagent III	Powder×1	4℃
Reagent IV	Powder×1	-20℃
Reagent V	Powder×2	-20℃
Reagent VI	Powder×2	-20℃

Solution preparation:

1. **Reagent II:** Dissolved with 0.5 mL of distilled water before use. Mix thoroughly.
2. **Reagent III:** Dissolved with 0.5 mL of distilled water before use. Mix thoroughly. It can be divided into small tubules and stored for 4 weeks at -20℃. Avoid repeating freeze/thaw cycles.
3. **Reagent IV:** Dissolved with 1.25 mL of distilled water before use. Mix thoroughly. It can be divided into small tubules and stored for 4 weeks at -20℃. Avoid repeating freeze/thaw cycles.
4. **Reagent V:** Dissolved with 1 mL of distilled water before use. Mix thoroughly. It can be divided into small tubules and stored for 4 weeks at -20℃. Avoid repeating freeze/thaw cycles.
5. **Reagent VI:** Dissolved with 1 mL of distilled water before use. Mix thoroughly. It can be divided into small tubules and stored for 4 weeks at -20℃. Avoid repeating freeze/thaw cycles.
6. **Working solution:** Calculate according to the amount required for the experiment before use, according to the ratio of reagent I: reagent II: reagent III: reagent IV: distilled water= 148μL: 4μL: 4μL: 4μL :10μL(amount of 1T), mix well before use.

Product Description：

Glycogen phosphorylase is divided into active glycogen phosphorylase a (Glycogen phosphorylase a, GPa) and inactive glycogen phosphorylase b (Glycogen phosphorylase b, GPb) two forms. The decomposition of glycogen is mainly carried out under the catalysis of glycogen phosphorylase a. When no activator is added, glycogen phosphorylase a catalyzes the production of glucose residues from glycogen and inorganic phosphorus to glycogen and glucose 1-phosphate. Under the action of phosphoglucose mutase and 6-phosphate glucose dehydrogenase, it further catalyzes the reduction of NADP to NADPH. Measuring the rate of increase of NADPH at 340nm can reflect the activity of glycogen phosphorylase a.

Reagents and Equipment Required but Not Provided：

Ultraviolet spectrophotometer/microplate reader, low temperature centrifuge, constant temperature incubator/water bath, adjustable pipette, mortar/homogenizer, micro quartz cuvette/96 well UV plate, ice and distilled water.

Procedure

I. Sample preparation:

1. Tissue sample: according to the proportion of tissue weight (g): extraction solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extraction reagent and fully homogenized on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.
2. Bacteria or cells: collecting bacteria or cells into the centrifuge tube, suggested 5 million with 1 mL of Extraction reagent. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 200w, working time 3 seconds, interval 10 seconds, 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.
3. Serum (plasma) sample: detect sample directly.

II. Determination procedure：

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.
2. Working solution are preheated at 37℃ for 5min.
3. GPa activity: add 10 μL sample, 10 μL reagent V, 10 μL reagent VI, 170 μL working solution in the micro quartz cuvette/96 well UV plate, mix immediately, and record the absorbance value at 10s at 340 nm A1, quickly place it in 37℃ water bath or incubator (The microplate reader has a temperature control function that can adjust the temperature to 37°C) for 10 minutes, take it out and quickly dry it and measure the absorbance value A2 in 10min10s.Calculate ΔA=A2-A1.Blank tube only need to be test one or two times.

III. Calculations：

（1）Micro glass cuvette

A. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every milligram protein in 37℃.

GPa（nmol/min/mg prot）＝[ΔA×V_R÷（ε×d）×10^9]÷(V_S×Cpr) ÷T=321.54×ΔA÷Cpr

B. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every gram tissue in 37℃.

GPa（nmol/min/g weight）＝[ΔA×V_R÷（ε×d）×10^9]÷(W×V_S÷V_E) ÷T=321.54×ΔA÷W

C. Sample volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every milliliter volume in 37℃.

GPa（nmol/min/mL）＝[ΔA×V_R÷（ε×d）×10^9]÷V_S ÷T=321.54×ΔA

D. Bacteria or cells number

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every million bacteria or cells in 37℃.

GPa（nmol/min/10^4 cells）＝[ΔA×V_R÷（ε×d）×10^9]÷(cell number×V_S÷V_E) ÷T=321.54×ΔA÷cell number

ε：NADPH molar extinction coefficient，6220 L/mol/cm；

d：Cuvette light path，1cm；

V_R: Total reaction volume, 0.2 mL;

V_S：Add sample volume,0.01mL；

V_E: extract volume, 1 mL;

W：Sample weight, g;

Cpr：Protein concentration of sample, mg/mL;

T：Reaction time，10min

（2）96-Well flat-bottom plates

Modify the d-1cm in the above formula to d-0.6cm (the light path of the 96-well plate) for calculation.

Note:

1. If the measured absorbance value ΔA>0.6, it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula; if the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement.

Experimental example

1. Take 0.1 g of rabbit liver tissue, add 1 mL of extract, homogenize in an ice bath, centrifuge at 8000 ×g for 10 minutes at 4℃; take the supernatant and place on ice for testing. Use micro quartz cuvette to operate according to the determination steps,ΔA=A2-A1=0.3472-0.2253=0.1219, according to the formula Calculated activity:

GPa（ nmol/min/g weight） ＝ [ΔA×V_R÷（ ε×d） ×10^9]÷(W ×V_S÷V_E)÷T=321.54×ΔA÷W=391.96 nmol/min/g weight