SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Sucrose Phosphorylase (SP)Activity Assay Kit

Note: Take two or three different samples for prediction before test.
Operation Equipment: Ultraviolet spectrophotometer/Microplate reader

Catalog Number: AK0338

Size: 100T/96S

Components:

Reagent	Size	Storage
Extract solution	110mL×1	4℃
Reagent I	7.5mL×1	4℃
Reagent II	Powder×1	-20℃
Reagent III	1mL×1	4℃
Reagent IV	Powder×2	-20℃
Reagent V	Powder×1	-20℃
Reagent VI	Powder×2	-20℃
Reagent VII	Powder×2	-20℃

Solution preparation:

1. Reagent II: Dissolved with 6 mL of distilled water before use. Mix thoroughly. It can be stored for 4 weeks at 4℃ .
2. Reagent IV：Dissolved with 0.6 mL of distilled water before use. Mix thoroughly. It can be stored for 2 weeks at -20℃ .
3. Reagent V：Dissolved with 10 mL of distilled water before use. Mix thoroughly. It can be stored for 4 weeks at -20℃ .
4. Reagent VI ： Dissolved with 1 mL of distilled water before use. Mix thoroughly. It can be divided into s mall tubules and preserved at -20℃ . Avoid repeating freeze/thaw cycles. It can be stored for 2 weeks at -20℃ . Before use, dilute the reagent VII according to the ratio of reagent VI: distilled water=1:1.
5. Reagent VII ： Dissolved with 0.7 mL of distilled water before use. Mix thoroughly. It can be divided i nto small tubules and preserved at -20℃ . It can be stored for 2 weeks at -20℃ . Before use, dilute the reagent VII according to the ratio of reagent VIII: distilled water=1:1.
6. **All reagents stored at -20℃ can be stored in aliquots to avoid repeated freezing and thawing**

Product Description：

Sucrose Phosphorylase (SP) (EC2.4. 1.7) mainly exists in microorganisms and plants. Sucrose phosphorylase cleaves the glucosidic bond and catalyzes the transfer of the glucosyl group to fructose, xylose, galactose and rhamnose to synthesize the corresponding glucosyl oligosaccharides. In addition, sucrose phosphorylase can also catalyze the synthesis of arbutin from hydroquinone, which has a strong whitening effect and has important applications in the cosmetics industry.

Sucrose phosphorylase can use phosphoric acid as the receptor to catalyze the production of glucose 1-phosphate from sucrose, which is transformed into glucose 6-phosphate under the catalysis of glucose

phosphate mutase, and reduces NADP+ to NADPH under the action of glucose 6-phosphate dehydrogenase. This results in an increase in light absorption at 340nm. The sucrose phosphorylase activity can be calculated by measuring the increase rate of absorbance at 340nm.

Reagents and Equipment Required but Not Provided：

Ultraviolet spectrophotometer/microplate reader, low temperature centrifuge, constant temperature incubator, adjustable pipette, mortar/homogenizer, micro quartz cuvette/96 well UV flat -bottom 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 10000 ×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 7 seconds, for 3 minutes). Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice for testing.

3. Liquid 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. Preheat Reagent I at 37℃ for 10 minutes.
3. Add reagents with the following list:

Reagent （μL）	Blank tube (A_B)	Test tube (A_T)
Reagent I	85	65
Reagent II	50	50
Reagent III	5	5
Reagent IV	10	10
Reagent V	10	10
Reagent VI	20	20
Reagent VII	20	20
Mix thoroughly ， 37℃ water bath preheating 5min.		
sample	-	20

Add the above reagents to the cuvette and quickly mix by pipetting， Record the absorbance value A_T 1（A_B 1） of the tube in 15s, 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 2 minutes, take it out and quickly dry it and measure the absorbance value A_T2（A_B2） in 2min15s , Calculate ΔA = (A_T2-A_T 1)-(A_B2- A_B 1).

Note: Blank tube only need to test 1-2 times. If the number of samples is too large, you can also mix Reagent 1 to Reagent 7 according to the above ratio and then perform the measurement.

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℃ .

SP (U/mg prot) ＝=ΔA÷ε÷d×V_R ×10^9÷V_S÷Cpr÷T =803.85×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℃ .

SP (U/g weight) =ΔA÷ε÷d×V_R ×10^9÷(V_S÷V_E ×W)÷T=803.85×ΔA÷W

C. Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every 10^4 bacteria or cells in 37℃ .

SP (U/10^4 cell)＝ΔA÷ε÷d×V_R ×10^9÷ (V_S÷V_E × cell numbers（10^4）)÷T=803.85×ΔA÷cell numbers（10^4）

χ： 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,0.02mL；
V_E : extract volume, 1 mL;
W： Sample weight, g;
Cpr：Protein concentration of sample, mg/mL;
T： Reaction time， 2min

（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 A>1, 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 potatoes, add 1 mL of extract, homogenize in an ice bath, centrifuge at 10000 ×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, calculate ΔA=（0.4070-0.3117）-（0.0956-0.0949）=0.0946, according to the formula Calculated activity:

SP activity (U/g weight) =803.85×ΔA÷W=760.44 U/ g weight

2. Take 0.1 g of black rice, add 1 mL of extract, homogenize in ice bath, 10000 g, Centrifuge at 10000 ×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, calculate ΔA =（0.4559-0.3546）-（0.0956-0.0949）=0. 1006, calculate the activity according to the formula:

SP activity（U/g weight）=803.85×ΔA÷W=808.67 U/ g weight

Related products

AK0538/AK0537 Sucrose Synthetase (SS) Activity Assay Kit

AK0285/AK0284 Neutral Invertase (NI) Activity Assay Kit

AK0287/AK0286 Acid Invertase (AI) Activity Assay Kit

AK0534/AK0533 Sucrose Phosphoric Acid Synthetase (SPS) Activity Assay Kit