Sucrose Phosphorylase (SP)Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0455

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 7.5 mL×1	2-8℃
Reagent II	Powder ×1	2-8℃
Reagent III	Liquid 1 mL×1	2-8℃
Reagent IV	Powder ×2	-20℃
ReagentV	Powder ×1	-20℃
ReagentVI	Powder ×2	-20℃
ReagentVII	Powder ×2	-20℃

Solution Preparation:

1. **Reagent II:** Dissolve it with 6 mL of distilled water before use. It can be stored for 4 weeks at 2-8℃.
2. **Reagent IV:** Dissolve one with 0.6 mL of distilled water before use. It can be sub-packaged and stored at -20℃ for 2 weeks. Avoid repeated freezing and thawing during storage.
3. **Reagent V:** Place the powder in a glass tube inside the bottle. Dissolve it with 10 mL of distilled water before use. It can be sub-packaged and stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing during storage.
4. **Reagent VI:** Before use, take one tube and add 1 mL of distilled water, mix well (can be used for 100T, to ensure the use time of the reagent kit, an extra tube is given); Can be packaged and stored at -20℃ to avoid repeated freezing and thawing. It can be stored for 2 weeks at -20℃; Before use, dilute the reagent VI according to the ratio of reagent VI: distilled water=1:1, ready for use.
5. **Reagent VII:** Dissolve one with 0.7 mL distilled water before use. It can be sub-package and store for 2 weeks at -20℃. Before use, dilute the reagent VII according to the ratio of reagent VII: distilled water=1:1, ready for use.

Note: Reagents VI and VII are freeze-dried reagents, and there may be significant or even small differences in the amount of reagents observed by the naked eye between different bottles. This phenomenon does not affect the use, and the actual quality is the same.

Product Description：

Sucrose phosphorylase (SP) (EC2.4.1.7) is mainly found in microorganisms and plants, belonging to

the glycosyl hydrolase 13 family. It is an enzyme that catalyzes the transfer of glucosidic bonds and can catalyze the synthesis of 1-phosphate glucose from sucrose and inorganic phosphate. This enzyme mainly uses sucrose and 1-phosphate glucose as donors, and various substances such as polyhydroxy sugars and sugar alcohols, phenolic hydroxyl groups, carboxyl groups, etc. as acceptors to catalyze the synthesis of various glycosides.

SP can catalyze sucrose to produce 1-phosphate glucose, which is then converted to 6-phosphate glucose by glucose phosphate mutase. Under the action of 6-phosphate glucose dehydrogenase, $NADP^+$ is reduced to form NADPH, resulting in an increase in 340nm light absorption value. The increase rate of 340nm absorbance is used to reflect SP activity.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, low temperature centrifuge, water bath/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 Extract 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 Extract 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. If the liquid is turbid, centrifuge and take the supernatant for measurement.

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_T1(A_B1) 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 $\Delta A = (A_T2-A_T1)-(A_B2-A_B1)$.

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

III. Calculations：

(1) Micro quartz 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)＝=$\Delta A \div \varepsilon \div d \times V_R \times 10^9 \div (V_S \times Cpr) \div T = 803.85 \times \Delta A \div Cpr$

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) =$\Delta A \div \varepsilon \div d \times V_R \times 10^9 \div (V_S \div V_E \times W) \div T = 803.85 \times \Delta A \div W$

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)＝$\Delta A \div \varepsilon \div d \times V_R \times 10^9 \div (V_S \div V_E \times N) \div T = 803.85 \times \Delta A \div N$

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

D: Cuvette light path, 1 cm；

V_R: Total reaction volume, 0.0002 L;

V_S: Add sample volume,0.02 mL；

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 solution, 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, calculateΔA= (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 solution, 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

BC0580/BC0585	Sucrose Synthetase (SS) Activity Assay Kit
BC0570/BC0575	Neutral Invertase (NI) Activity Assay Kit
BC0560/BC0565	Acid Invertase (AI) Activity Assay Kit
BC0600/BC0605	Sucrose Phosphoric Acid Synthetase (SPS) Activity Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com