（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K1205-M

Specification：48T(48 samples)/96T(96 samples)

Measuring instrument：Microplate reader(340 nm)

Detection range：24-1400 U/g

Elabscience® Starch Phosphorylase (SP) Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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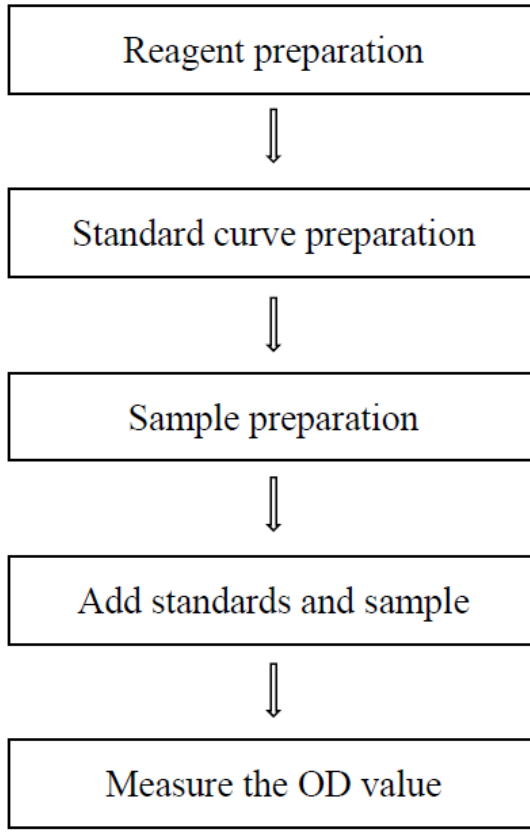
Assay summary



Intended use

This kit can be used to measure starch phosphorylase (SP) activity in plant tissue samples.

Detection principle

Starch Phosphorylase (SP) breaks down the substrate to produce α-D-glucose-1 phosphate, which is converted to α-D-glucose-6 phosphate by the enzyme. It also reduces NAD^+/$NADP^+$ with the catalysis of dehydrogenase to produce NADH/NADPH, and the absorbance value at 340 nm increases.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Extraction Solution	55 mL × 1 vial	55 mL × 2 vials	2-8 ℃, 12 months
Reagent 2	Reaction Solution	6 mL × 1 vial	12 mL × 1 vial	2-8 ℃, 12 months, shading light
Reagent 3	Substrate A	Powder × 1 vial	Powder × 2 vials	2-8 ℃, 12 months, shading light
Reagent 4	Substrate B	Powder × 1 vial	Powder × 2 vials	2-8 ℃, 12 months, shading light
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator (25℃)

Regent：

Ultrapure water

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of substrate A working solution:

Dissolve one vial of substrate A with 2 mL of ultrapure water, mix well to dissolve. Aliquoted storage at -20 ℃ for a month.

③ The preparation of substrate B working solution:

Dissolve one vial of substrate B with 2 mL of ultrapure water, mix well to dissolve. Aliquoted storage at -20 ℃ for a month.

④ The preparation of reaction working solution:

For each well, prepare 180 μL of reaction working solution (mix well 108 μL of reaction solution, 36 μL of substrate A working solution and 36 μL of substrate B working solution). The reaction working solution should be prepared on spot and the prepared solution should be used up within 0.5 h.

Sample preparation

① Sample preparation:

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 0.1 g).

② Homogenize 0.1 g tissue in 0.9 mL extraction solution with a dounce homogenizer at 4℃.

③ Centrifuge at 15000 ×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Potato tissue homogenate	1
10% Corn tissue homogenate	1
10% Pumpkin seed tissue homogenate	1
10% Garlic tissue homogenate	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The testing time should be strictly controlled, and it's better to measure no more than 10 sample wells at same time.

② The reaction working solution should be prepared on the spot, and it is recommended to prepare it before detection.

Operating steps

① Sample well: Add 20 μL of sample to the corresponding well

② Add 180 μL of reaction working solution to each well.

③ Mix fully with microplate reader for 5 s and incubate at 25℃ for 3 min. Measure the OD value of each well at 340 nm with microplate reader, as A_1. Incubate at 25℃ for 3 min, measure the OD value of each well at 340 nm with microplate reader, as A_2, $\Delta A = A_2 - A_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue samples (Calulate for sample weight):

Definition: The amount of enzyme in 1 g of tissue that produce 1 nmol NADPH at 37 ℃ for 1 min is defined as 1 unit.

$$\text{SP activity (U/g)} = \frac{\Delta A}{\varepsilon \times d} \times V_1 \times 10^{\wedge 9} \div T \div (m \times \frac{V_2}{V_3}) \times f$$

$$= \Delta A \div m \times f \times 482.31$$

Tissue samples (Calulate for protein concentration):

Definition: The amount of enzyme in 1 mg of tissue protein that produce 1 nmol NADPH at 37 ℃ for 1 min is defined as 1 unit.

$$\text{SP activity (U/mgprot)} = \frac{\Delta A}{\varepsilon \times d} \times V_1 \times 10^{\wedge 9} \div T \div (C_{pr} \times V_2) \times f$$

$$= \Delta A \div C_{pr} \times f \times 535.9$$

[Note]

ΔA: $A_2 - A_1$

ε: The molar extinction coefficient of at 340 nm, 6220 L/mol/cm

d: Optical path, 0.6 cm.

V_1: The volume of reaction system, 0.0002 L

$10^{\wedge 9}$：1 mol = $10^{\wedge 9}$ nmol

T：Reaction time，5 min

m：The weight of sample, g

V_2：The volume of sample added to the reaction system, 0.02 mL

V_3：The volume of the extraction solution during sample disposing, mL

f：Dilution factor of sample before test

C_{pr}: Concentration of protein in sample, mgprot/mL

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three corn samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g)	220	340	450
%CV	5.3	4.2	3.7

Inter-assay Precision

Three corn samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g)	220	340	450
%CV	4.2	5.2	5.8

Sensitivity

The analytical sensitivity of the assay is 24 U/g. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

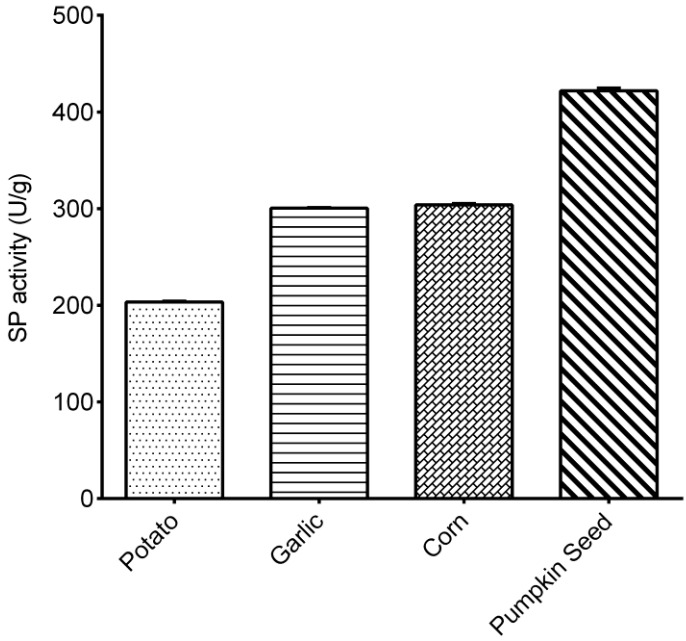
Example analysis:

Take 20 μL of 10% potato tissue homogenate supernatant and inactivated sample, carry the assay according to the operation steps. The results are as follows:

The average OD value of the A_1 is 0.407, the average OD value of the A_2 is 0.362, and the calculation result is:

$$\text{SP activity (U/g)} = (0.407\text{-}0.362) \div 0.1 \times 1 \times 482.31 = 217.04 \text{ U/g}$$

Detect 10% potato tissue homogenate, 10% corn tissue homogenate, 10% garlic tissue homogenate, 10% pumpkin seed tissue homogenate, according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.