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

Catalog No：E-BC-K1203-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader（340 nm）

Detection range：83-6640 U/g wet weight

Elabscience® Granule-Bound Starch Synthase (GBSS) 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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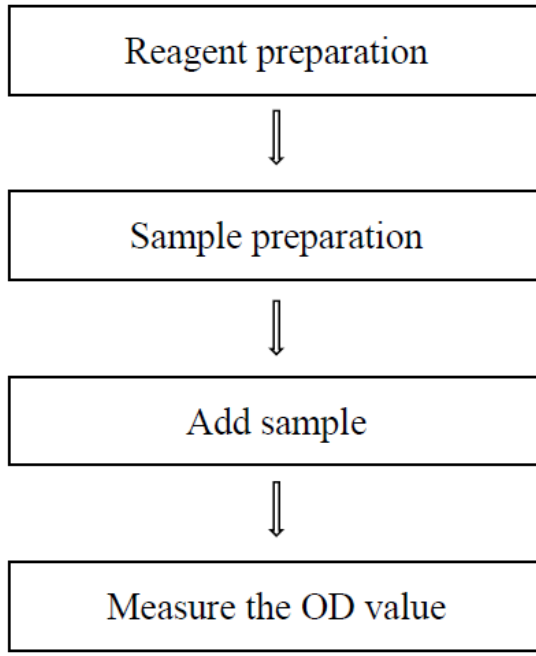
Assay summary



Intended use

This kit can be used to measure granule-bound starch synthase (GBSS) activity in plant tissue sample.

Detection principle

Granule-bound Starch Synthase (GBSS) catalyzes the reaction between ADPG and the primer, transferring glucose molecules to the primer and generating ADP, which in turn catalyzes the reduction of $NADP^+$ to NADPH. The amount of NADPH produced was proportional to the amount of ADP produced in the previous reaction step, and GBSS activity was calculated by measuring the increase in NADPH at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	60 mL ×1 vial	60 mL ×2 vials	-20℃, 12 months
Reagent 2	Buffer Solution	10 mL ×1 vial	20 mL ×1 vial	-20℃, 12 months
Reagent 3	Substrate	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months
Reagent 4	Enzyme Reagent	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months
Reagent 5	Chromogenic Agent A	2.5 mL ×1 vial	4.5 mL ×1 vial	-20℃, 12 months
Reagent 6	Chromogenic Agent B	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months
Reagent 7	Chromogenic Agent C	Powder ×1 vial	Powder ×2 vials	-20℃, 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), Homogenizer, Water bath, Centrifuge, Incubator.

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of substrate working solution:
Dissolve a vial of substrate with 5.5 mL buffer solution and mix well.
Aliquoted storage at -20 ℃ for a month, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of enzyme working solution:
Dissolve a vial of enzyme reagent with 3 mL buffer solution and mix well.
Aliquoted storage at -20 ℃ for a month, and avoid repeated freeze/thaw cycles is advised.

④ The preparation of chromogenic agent B working solution:
Dissolve a vial of chromogenic agent B with 0.15 mL buffer solution and mix well. Aliquoted storage at -20 ℃ for a month, and avoid repeated freeze/thaw cycles is advised.

⑤ The preparation of chromogenic agent C working solution:
Dissolve a vial of chromogenic agent C with 0.15 mL buffer solution and mix well. Aliquoted storage at -20 ℃ for a month, and avoid repeated freeze/thaw cycles is advised.

⑥ The preparation of chromogenic working solution：

For each well, prepare 90 μL of chromogenic working solution (mix well 80 μL of chromogenic agent A, 5 μL of chromogenic agent B working solution and 5 μL of chromogenic agent C working solution). The chromogenic working solution should be prepared on spot, and the prepared solution should be used up within 20 min.

Sample preparation

① Sample preparation

Plant Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×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).

Control sample:

Take 0.3 mL of supernatant for detection to the new EP tubes, boiling water bath for 5 min, cool down with running water, as control sample.

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% Sweet potato 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 sample homogenization process was kept at a low temperature and fully homogenized, and mixed with pipette before use.

② The chromogenic reaction rate is fast, and it's better to measure no more than 20 sample wells at same time.

Operating steps

Enzyme reaction:

① Control well: Add 50 μL of sample to the corresponding 1.5 mL EP tube.
Sample well: Add 50 μL of sample to the corresponding 1.5 mL EP tube.

② Add 90 μL of substrate working solution to each tube.

③ Mix fully and incubate at 30℃ for 20 min, boil water bath for 1 min (cover tightly to prevent water loss), cool down with running water.

④ Add 50 μL of enzyme working solution to each tube.

⑤ Mix fully and incubate at 30℃ for 30 min, boil water bath for 1 min (cover tightly to prevent water loss), cool down with running water. Centrifuge at 10000 × g for 10 min at 4℃.

Chromogenic reaction:

① Take 100 μL of supernatant into the UV-Microplate, add 45 μL of chromogenic working solution

② Mix fully and incubate at 30℃ for 5 min. Measure the OD value of each well with microplate reader at 340 nm.

Calculation

The sample:

The plant tissue sample:

Definition: The amount of enzyme in 1 g tissue protein that catalyze the substrate to 1 μmol NADPH in 1 h at 30℃ is defined as 1 unit.

$$\text{GBSS activity (U/g wet weight)} = \frac{\Delta A \times V_1}{\varepsilon \times d} \times 10\text{^}9 \times f \div (V_2 \div V_3 \times m) \div T \times 1.9^*$$

$$= 3322 \times \Delta A \div m \times V_3 \times f$$

[Note]

ΔA: $OD_{sample} - OD_{control.}$

V_1: The volume of reaction system, 0.145×10^-3 L

ε: Molar absorption coefficient, 6.22×10^3 L/(mol*cm)

d: Optical path, 0.4 cm

V_2: The volume of sample, 0.05 mL

V_3: The volume of extraction solution, mL

T: The time of reaction, 2/3 h

*: Dilution of the reaction system

m: The weight of sample, g

10^9: 1 mol = 10^9 nmol

f: Dilution factor of sample before test

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three potato tissue 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 wet weight)	0.3	0.4	0.5
%CV	1.4	1.8	3.1

Inter-assay Precision

Three potato samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g wet weight)	0.3	0.4	0.5
%CV	3.5	6.3	2.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(U/g wet weight)	3093.1	4327.1	5284.3
Observed Conc.(U/g wet weight)	3130.6	4262.6	5303.7
Recovery rate (%)	101.2	98.5	100.4

Sensitivity

The analytical sensitivity of the assay is 83 U/g wet weight. 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 II Example Analysis

Example analysis：

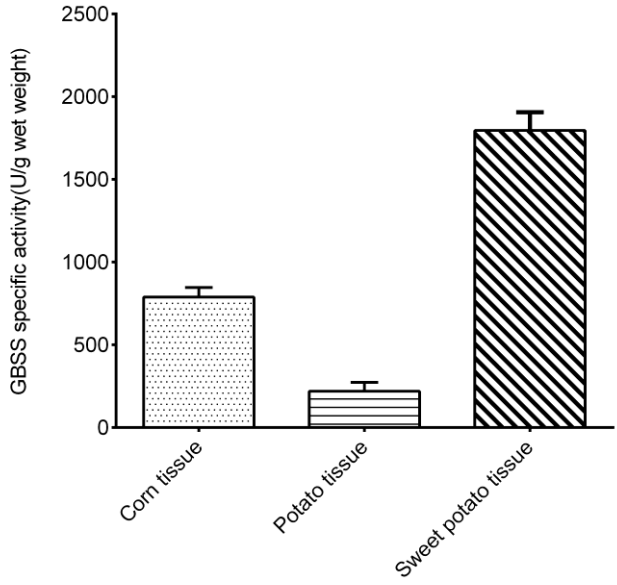
Take 50 μL of 10% corn tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

The OD value of the sample well is 0.238, the OD value of the control well is 0.214, ΔA =0.238-0.214=0.024, and the calculation result is:

$$\text{GBSS activity (U/g wet weight)} = 0.024 \times 3322 \div 0.1 \times 0.9$$

$$= 717.5 \text{ U/g wet weight}$$

Detect 10% corn tissue homogenate, 10% potato tissue homogenate, 10% sweet potato 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.