

Soluble Starch Synthase (SSS) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC1850

Size: 50T/48S

Components:

Extract solution: Liquid 60 mL×1, store at 2-8°C.

Reagent I: Liquid 16 mL×1, store at 2-8°C.

Reagent II A: Powder×2, store at 2-8°C.

Reagent II B: Powder×2, store at -20°C.

Reagent II C: Powder×2, store at -20°C.

Reagent II prepares: Before use, take a bottle of **Reagent II A**, add 8mL of **Reagent I**, heat it slowly, gradually raise the temperature to boil and to dissolve it. And then add **1 Reagent II B** and **1 Reagent II C** to mix and dissolve it after cooling. The unused reagent shall be sub packed and stored at - 20°C for 2 weeks. Avoid repeated freezing and thawing.

Reagent III A: Liquid 12 mL×1, store at 2-8°C.

Reagent III B: Powder×2, store at -20°C.

Reagent III prepares: Take a bottle of **Reagent III B**, add 5mL of **Reagent III A**, mix thoroughly, the unused reagent shall be sub packed and stored at - 20°C for 4 weeks. Avoid repeated freezing and thawing.

Reagent IV: Liquid 30 μ L×1, store at 2-8°C. Centrifugation before use, take 13 μ L of Reagent IV, add 4.16mL of Reagent III to mix up (about for 27 tubes). It can also be prepared in proportion according to the actual sample size.

Reagent V-A: Liquid 18 mL×1, store at 2-8°C.

Reagent V-B: Powder×2, store at 2-8°C.

Reagent V-C: Powder×2, store at 2-8°C.

Reagent V prepares: Before use, take a bottle of Reagent **V-B**, add 8mL of Reagent V-A and a bottle Reagent V-C to dissolve. The unused reagent shall be sub packed and stored at - 20°C for 4 weeks. Avoid repeated freezing and thawing.

Reagent VI: Powder×4, store at -20°C. Take a bottle of Reagent VI, add 208 μ L distilled water before use, mix thoroughly. The unused reagent shall be sub packed and stored at - 20°C for 2 weeks. Avoid repeated freezing and thawing.

Reagent VII: Powder×1, store at -20°C. Add 2mL distilled water before use, mix thoroughly. The unused reagent shall be sub packed and stored at - 20°C for 8 weeks. Avoid repeated freezing and thawing.

Product Description:

Soluble Starch Synthase (SSS, EC 2.4.1.21) usually present in the free matrix in the plastid

matrix, which catalyzes the elongation of the starch chain, mainly responsible for the synthesis of amylopectin.

SSS catalyzes the reaction of ADPG with starch primer (glucan), transfers glucose molecules to starch primers, and simultaneously produces ADP. Add pyruvate kinase, hexokinase and 6-phosphate glucose dehydrogenase to the reaction system. These enzymes in turn catalyze NADP⁺ reduction to NADPH, the amount of NADPH produced is proportional to the amount of ADP produced in the previous step reaction, and the SSS activity can be calculated by measuring the increase of NADPH at 340 nm.

Required but not provided:

Ultraviolet Spectrophotometer, Water Bath, Desk Centrifuge, Transferpettor, 1 mL Quartz Cuvette, Mortar/Homogenizer, Ice and Distilled Water.

Protocol:

I. Sample Preparation.

Add 1 mL Extract solution to 0.1 g tissue, homogenate on ice. 10000 g centrifuge at 4°C for 10 min. Take the supernatant on ice for test.

II. Determination procedure.

1. Preheat spectrophotometer for 30 min, adjust wavelength to 340 nm, set zero with distilled water.
2. Add reagents to centrifuge tube according to the following table.

Reagent Name (μL)	Test Tube (V _T)
Sample	200
Reagent II	270
Mix thoroughly, keep warm for 20 min at 30°C, place at boiled water for 1 min (cover tightly to prevent water loss), cold on ice.	
Reagent IV	150
Mix thoroughly, keep warm for 30 min at 30°C, place at boiled water for 1 min (cover tightly to prevent water loss), cold on ice. 10000 g centrifuge for 10 min at room temperature. Take supernatant. Preheat Reagent V and supernatant at 37°C.	
Supernatant	450
Reagent V	300
Reagent VI	15
Reagent VII	15

Mix thoroughly. Measure the absorbance at 340 nm. Record the initial absorbance value A₁, after 2 min's reaction record absorbance value A₂. Calculate $\Delta A = A_2 - A_1$.

Note: If Reagent II had precipitation, mix thoroughly before add.

III. Calculation

1. Sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADPH in the reaction system per minute every mg protein.

$$SSS (U/mg \text{ prot}) = [\Delta A \div (\epsilon \times d) \times V_T] \div (Cpr \times V_{SA} \div V_{RT} \times V_S) \div T = 43.2 \times \Delta A \div Cpr$$

This method needs to determine the protein concentration of crude enzyme solution.

2. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of NADPH in the reaction system per minute every g sample.

$$SSS (U/g \text{ weight}) = [\Delta A \div (\epsilon \times d) \times V_T] \div (W \div V_E \times V_{SA} \div V_{RT} \times V_S) \div T = 43.2 \times \Delta A \div W$$

V_T : Test volume, 0.78 mL.

V_{RT} : React solution volume, 0.62 mL .

V_E : Extract solution volume, 1 mL.

T: Reaction time, 20 min.

ϵ : The molar extinction coefficient of NADPH, 6.22×10^{-3} mL/(nmol·cm).

d: The optical path of cuvette, 1 cm.

V_{SA} : Sample volume, 0.2 mL.

V_S : Supernatant volume, 0.45 mL.

Cpr: Sample protein concentration, mg/mL.

W: Sample weight, g.

Experimental example:

1. Take 0.1g liver to 1mL extract solution, grinding on ice, 10000 rpm centrifuge at 4°C for 10 min, supernatant is ready for test, operate as the procedure, $\Delta A = A_2 - A_1 = 0.394 - 0.211 = 0.183$, calculate content by sample weight: $SSS (U/g \text{ weight}) = 43.2 \times \Delta A \div W = 79.056 U/g \text{ weight}$.
2. Take 0.1g Ilex to 1mL extract solution, grinding on ice, 10000 rpm centrifuge at 4°C for 10 min, supernatant is ready for test, operate as the procedure, $\Delta A = A_2 - A_1 = 1.31 - 1.303 = 0.007$, calculate content by sample weight: $SSS (U/g \text{ weight}) = 43.2 \times \Delta A \div W = 3.024 U/g \text{ weight}$.

References:

[1] Jiang H, Dian W, Wu P. Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme[J]. Phytochemistry, 2003, 63(1): 53-59.

Related products:

BC3290/BC3295 Bound Station Amylosynthase (GBSS)Activity Assay Kit

BC1860/BC1865 Starch Branching Enzyme(SBE) Activity Assay Kit