Soluble Starch Synthase(SSS) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC1855

Size: 100T/96S

Components:

Extract solution: Liquid 110 mL×1, store at 2-8℃.

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

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

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

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

Reagent II prepared: 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. Then add a bottle of Reagent II B and a bottle of Reagent II C to mix and dissolve it after cooling. The unused reagent shall be sub packed and stored at - 20℃ for 2 weeks. Avoid repeated freezing and thawing.

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

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

Reagent III prepared: 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℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent IV: Liquid 27 μL×1, store at 2-8℃. Centrifugation before use, take 12.5μL of Reagent IV, add 4mL of Reagent III to mix up (about for 53 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℃.

Reagent V-B: Powder×2, store at 2-8℃.

Reagent V-C: Powder×2, store at 2-8℃.

Reagent V prepared: Before use, mix and dissolve a bottle of Rreagent V-B, a bottle of Rreagent V-C and 8mL of Rreagent V-A. The unused reagent shall be sub packed and stored at - 20℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent VI: Powder×3, store at -20℃. Add 208 μL distilled water with one bottle before use, mix thoroughly. The unused reagent shall be sub packed and stored at - 20℃ for 2 weeks. Avoid repeated freezing and thawing.

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

Product Description:

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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:

Spectrophotometer/Microplate Reader, Water Bath, Desk Centrifuge, Transferpettor, Micro Quartz Cuvette/96 Well UV Plate, 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℃ for 10 min. Take the supernatant on ice for test.

II. Determination procedure.

1. Preheat Spectrophotometer or microplate reader for 30 min, adjust wavelength to 340 nm. The spectrophotometer needs to be zeroed with distilled water.
2. Add reagents to centrifuge tube according to the following table.

Reagent Name (μL)	Test Tube (V_T)
Sample	100
Reagent II	135
Mix thoroughly, keep warm for 20 min at 30℃, place at boiled water for 1 min (cover tightly to prevent water loss), cold on ice.	
Reagent IV	75
Mix thoroughly, keep warm for 30 min at 30℃, 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℃.	
Supernatant	150
Reagent V	100
Reagent VI	5
Reagent VII	5

Immediately take out 200 μL working solution to micro quartz cuvette or 96 well UV plate after mix thoroughly. Measure the absorbance at 340 nm. Record the initial absorbance value A1, after 2 mins reaction record absorbance value A2. Calculate ΔA=A2-A1.

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

III. Calculation

A. Micro quartz cuvette

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 prot)=[ΔA÷(ε×d)×V_T]÷(Cpr×V_{SA}÷V_{RT}×V_S) ÷T =43.2×ΔA÷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 weight)=[ΔA÷(ε×d)×VT]÷(W÷V_E×V_{SA}÷V_{RT}×V_S) ÷T =43.2×ΔA÷W

V_T: Test volume, 0.26 mL.

V_{SA}: Sample volume, 0.1 mL.

V_{RT}: React solution volume, 0.31 mL .

V_E: Extract solution volume, 1×10^{-3} L.

Vs: Supernatant volume, 0.15 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.

Cpr: Sample protein concentration, mg/mL.

W: Sample weight, g.

B. 96 well UV plate

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 prot)=[ΔA÷(ε×d)×V_T]÷(Cpr×V_{SA}÷V_{RT}×V_S) ÷T =72×ΔA÷Cpr

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 weight)=[ΔA÷(ε×d)×V_T]÷(W÷V_E×V_{SA}÷V_{RT}×V_S) ÷T=72×ΔA÷W

V_T: Test volume, 0.26 mL.

V_{SA}: Sample volume, 0.1 mL.

V_{RT}: React solution volume, 0.31 mL .

V_E: Extract solution volume, 1×10^{-3} L.

Vs: Supernatant volume, 0.15 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, 0.6 cm.

Cpr: Sample protein concentration, mg/mL.

W: Sample weight, g.

Experimental example:

1. Take 0.1g liver to 1mL extract solution, grinding on ice, 10000g centrifuge at 4℃ for 10 min, supernatant is ready for test, operate as the procedure, Δ A=A2-A1=0.3809-0.1959=0.185, calculate content by sample weight: SSS（U/g weight)= 43.2×ΔA÷W=79.92 U/g weight.
2. Take 0.1g Ilex to 1mL extract solution, grinding on ice, 10000g centrifuge at 4℃ for 10 min, supernatant is ready for test, operate as the procedure, Δ A=A2-A1=1.3654-1.3601=0.0053, calculate content by sample weight: SSS（U/g weight)= 43.2×ΔA÷W=2.2896 U/g 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 Amylosynthease (GBSS)Activity Assay Kit

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