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: NA0493

Size: 100T/96S

Components:

Extract solution: Liquid 100 mL×1, store at 4℃.

Reagent I: Liquid 35 mL×1, store at 4℃.

Reagent II: Powder×1, store at 4℃.

Reagent III: Powder×1, store at -20℃.

Reagent IV: Powder×2, store at -20℃. Add 5 mL Reagent I before use.

Reagent V: Powder×1, store at 4℃. Add 10 mL Reagent I before use.

Reagent VI: Powder×3, store at -20℃. Add 208 μL distilled water before use. Mix thoroughly. Surplus solution store at 4℃.

Reagent VII: Liquid 250 μL×2, store at -20℃ after spacing out. Avoid repeated freezing and thawing.

Reagent VIII: Liquid 12.5 μL×2. Add 4 mL dissolved Reagent IV before use.

Reaction solution I: Add 14 mL Reagent I to Reagent II, heat slowly make it dissolve, add Reagent III after cooled. It can be prepared in two batches and determined.

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:

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, set zero with distilled water.

2. Add reagents to centrifuge tube according to the following table.

Reagent Name (μL)	Test tube (V_T)
Sample	100
Reaction solution I	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 VIII	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. (If there are more samples for one-time determination, Reagent IV, V and VI can be proportionally mixed into a mixture.) 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 min's 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.

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

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.

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

V_T: Test volume, 0.26 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.

$SSS(U/mg\ prot)=[\Delta A\div(\varepsilon\times d)\times V_T]\div(Cpr\times V_{SA}\div V_{RT}\times V_S)\div T=72\times\Delta A\div Cpr$

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.

$SSS(U/g\ weight)=[\Delta A\div(\varepsilon\times d)\times V_T]\div(W\div V_E\times V_{SA}\div V_{RT}\times V_S)\div T=72\times\Delta A\div W$

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

NA0636/NA0394 Bound Station Amylosynthease (GBSS)Activity Assay Kit

NA0734/NA0492 Starch Branching Enzyme(SBE) Activity Assay Kit