Granule-Bound Starch Synthase (GBSS) Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC3295

Size:100T/96S

Components:

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

Reagent I: Liquid 16mL×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℃.

Preparation of Reagent II: 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 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 12mL×1，store at 2-8℃.

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

Preparation of Reagent III: Add 5 mL of Reagent III A into a bottle of Reagent III B and dissolve it fully before use. The unused reagent shall be sub packed and stored at - 20℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent IV: Liquid 27μL×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 18mL×1，store at 2-8℃.

Reagent V B: Powder×2，store at 2-8℃.

Reagent V C: Powder×2，store at 2-8℃.

Preparation of Reagent V: Before use, mix and dissolve a bottle of Reagent V B, a bottle of Reagent V C and 8mL of Reagent 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℃. Before use, take a bottle of Reagent VI, add 208 μL distilled water 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.

Description:

Granule-Bound Starch Synthase (GBSS， EC 2.4.1.21) is present in the amyloid body in a

bound state, catalyzing the elongation reaction of the starch chain, and is mainly responsible for the synthesis of amylose.

GBSS catalyzes the reaction of ADPG with starch primer (glucan), transferring glucose molecules to starch primers, and simultaneously generating ADP. Further, the pyruvate kinase, hexokinase and glucose-6-phosphate dehydrogenase added in the reaction system sequentially catalyze the reduction of $NADP^+$ to NADPH, wherein the amount of NADPH is proportional to the amount of ADP produced by the previous reaction, and the NADPH is measured at 340 nm. Increase the amount to calculate GBSS activity.

Required but not provided:

Spectrophotometer/Microplate Reader, Water Bath, Centrifuge, Transferpettor, Micro Quartz Cuvette/96 Well Flat-Bottom Plate (UV plate), Mortar, Ice and Distilled Water.

Protocol:

I. Sample Preparation.

Add 1mL of Extract solution to 0.1g of tissue, homogenate on ice bath, centrifuge at 10000g for 10min at 4℃, discard supernatant, add 1ml of extract solution to precipitation and mix thoroughly. To be tested on ice.

II. Preheat the spectrophotometer for 30min, adjust wavelength to 340 nm, set zero with distilled water.

III. Test procedure

Add following reagents in centrifuge tube.

Reagent Name(μL)	Tested Tube
Sample	100
Reagent II	135
Mix thoroughly. Place at 30℃ for 20min. Place on boiled water for 1 min, cool on ice.	
Reagent IV	75
Mix thoroughly. Place at 30℃ for 30min. Place on boiled water for 1 min, cool on ice. Centrifuge at 10000g at room temperature for 10min, take supernatant. 37℃ preheat reagent V and supernatant.	
Supernatant	150
Reagent V	100
Reagent VI	5
Reagent VII	5

Mix thoroughly. Take 200 μL into micro quartz cuvette/96 well plate (UV plate). Record the initial absorbance A1, after 2 min's reaction record absorbance value A2. ΔA=A2-A1.

Note: If reagent II had precipitation, mix thoroughly before added.

IV. GBSS activity calculation

A. micro quartz cuvette

1. Sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol of NADPH per minute every mg tissue protein

GBSS (U/mg prot)=[ΔA÷(ε× d)×Vt]÷(Cpr× Vs÷ Vrt × Vsp) ÷T =43.2×ΔA÷Cpr

2. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol of NADPH per minute every gram tissue weight

GBSS (U/g)=[ΔA÷(ε× d)×Vt×10^9]÷(W÷ Ve× Vs÷ Vrt× Vsp) ÷T=43.2×ΔA÷W

Vt: Test volume, 0.26mL

Vs: Sample volume, 0.1m L

Vrt: Total reaction volume, 0.31mL

Vsp: Supernatant volume, 0.15mL

Ve: Extraction solution volume, 1×10^{-3} L

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

d: The optical path of cuvette, 1cm

T: Reaction time, 20min

Cpr: Concentration of sample protein, mg/mL

W: Sample weight, g

B. 96 well plate

1. Sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol of NADPH per minute every mg tissue protein

GBSS (U/mg prot)=[ΔA÷(ε×d)×Vt]÷(Cpr×Vs÷Vrt ×Vsp) ÷T =72×ΔA÷Cpr

2. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol of NADPH per minute every gram tissue weight

GBSS (U/g)=[ΔA÷(ε×d)×Vt]÷(W÷Ve×Vs÷Vrt×Vsp) ÷T=72×ΔA÷W

Vt: Test volume, 0.26mL

Vs: Sample volume, 0.1m L

Vrt: Total reaction volume, 0.31mL

Vsp: Supernatant volume, 0.15mL

Ve: Extraction solution volume, 1×10^{-3} L

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

d: The optical path of 96 well plate, 0.6 cm
T:Reaction time, 20min
Cpr: Concentration of sample protein, mg/mL
W: Sample weight, g

Experimental example:

1. Take 0.1g liver, add 1 ml extract solution and homogenize in ice bath. centrifugation at 4℃ and 10000g for 10 min, discard the supernatant, add 1 ml of extract solution into the precipitation, mix well, and place on ice. Then operate according to the determination steps, calculate ΔA = A2-A1 = 0. 2685-0. 2532 = 0

GBSS activity (U/g mass) = 43.2×ΔA ÷ W = 6.6096 U/g mass.

2. Take 0.1g willow and add 1ml extract solution, homogenize in ice bath. centrifugation at 4℃ and 10000g for 10 min, discard the supernatant, add 1 ml extract solution into the precipitation, mix well, and put it on ice. Then, operate according to the determination steps, measure and calculate with micro quartz cuvette ΔA =A2-A1 = 2.2252-2.2184 =0.0068, and calculate the enzyme activity according to the sample mass

GBSS activity (U/g mass) = 43.2 × ΔA÷W = 2.9376 U/g mass.

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：

BC0700/BC0705	Starch Content Assay Kit
BC1850/BC1855	Soluble Starch Synthase(SSS) Activity Assay Kit
BC1860/BC1865	Starch Branching Enzyme(SBE) Activity Assay Kit