Granule-Bound Starch Synthase (GBSS) Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: NA0636

Size:50T/48S

Components:

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

Reagent I: Liquid 40mL×1，store at 4℃;

Reagent II: Powder×2，store at 4℃. Add 7mL of reagent I before use.

Reagent III: Powder×2，store at -20℃;

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

Reagent V: Powder×2，store at -20℃. Add 8mL of reagent I before use.

Reagent VI: Powder×4，store at -20℃. Add 208μL of distilled water before use, mix thoroughly. Surplus liquid store at 4℃;

Reagent VII: Liquid 250μL×3, store at -20℃;

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

Prepare Working solution I: Add 7mL of reagent I to reagent II before use, heat slowly. Gradually heat-up make it dissolve. Add reagent III after cold, mix thoroughly.

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, water bath, centrifuge, transferpettor, 1mL cuvette, mortar, ice and distilled water.

Protocol:

I. Sample Preparation.

Add 1mL of Extract solution to 0.1g of tissue, homogenate in ice bath, centrifuge at 10000g for 10min at 4℃, discard supernatant. add 1ml of extract solution to precipitation, 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 (μL)	Tested tube
Sample	200
Working solution I	270
Mix thoroughly. Place at 30℃ for 20min. Place on boiled water 1 min, cooled on ice.	
Reagent VIII	150
Mix thoroughly. Place at 30℃ for 30min. Place on boiled water 1 min, cooled on ice. Centrifuge at 10000g at room temperature for 10min, take supernatant. preheat reagent V and supernatant at 37℃.	
Supernatant	450
Reagent V	300
Reagent VI	15
Reagent VII	15

Mix thoroughly. 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

V. 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

VI. 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=43.2×ΔA÷W.

Vt: Test volume, 0.78mL

Vrt: Reaction total volume, 0.62mL

Ve: Extraction solution volume, 1mL

T: Reaction time, 20min

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

d: The optical path of cuvette, 1cm

Vs: Sample volume, 0.2mL

Vsp: Supernatant volume, 0.45mL

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=0.19-0.178=0.012
GBSS activity (U/g mass) =43.2×ΔA÷W=5.184 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 = 1.919-1.915=0.004, and calculate the enzyme activity according to the sample mass
GBSS activity (U/g mass) = 43.2×ΔA÷W=1.728 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：

NA0813/NA0571 Starch Content Assay Kit
NA0735/NA0493 Soluble Starch Synthase (SSS) Activity Assay Kit
NA0734/NA0492 Starch Branching Enzyme (SBE) Activity Assay Kit