

## Granule-Bound Starch Synthase (GBSS) Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Cat No:** BC3290

**Size:** 50T/48S

### Components:

**Extract solution:** Liquid 110mL×1, store at 2-8°C.

**Reagent I:** Liquid 16mL×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.

**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°C for 2 weeks. Avoid repeated freezing and thawing.

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

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

**Preparation of Reagent III:** Before use, take a bottle of Reagent III B, add 5mL of Reagent III A, dissolve it fully. The unused reagent shall be sub packed and stored at - 20°C for 4 weeks. Avoid repeated freezing and thawing.

**Reagent IV:** Liquid 30μL×1, store at 2-8°C. Centrifugation before use, take 13μL 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 18mL×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.

**Preparation of Reagent V:** Before use, mix and dissolve 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°C for 4 weeks. Avoid repeated freezing and thawing.

**Reagent VI:** Powder×4, store at -20°C. 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°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.

### 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  $\text{NADP}^+$  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°C, 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 Name (μL)	Tested Tube
Sample	200
Reagent II	270
Mix thoroughly. Place at 30°C for 20min. Place on boiled water 1 min, cooled on ice.	
Reagent IV	150
Mix thoroughly. Place at 30°C 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°C.	
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.  $\Delta A = A2 - A1$ .

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

**IV. GBSS activity calculation**

(1) 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

$$\text{GBSS (U/mg prot)} = [\Delta A \div (\epsilon \times d) \times V_t] \div (\text{Cpr} \times V_s \div V_{rt} \times V_{sp}) \div T = 43.2 \times \Delta A \div \text{Cpr}$$

(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

$$\text{GBSS (U/g)} = [\Delta A \div (\epsilon \times d) \times V_t] \div (W \div V_e \times V_s \div V_{rt} \times V_{sp}) \div T = 43.2 \times \Delta A \div W.$$

V<sub>t</sub>: Test volume, 0.78mL

V<sub>rt</sub>: Reaction total volume, 0.62mL

V<sub>e</sub>: Extraction solution volume, 1mL

T: Reaction time, 20min

ε: the molar extinction coefficient of NADPH,  $6.22 \times 10^{-3} \text{ mL}/(\text{nmol} \cdot \text{cm})$

d: The optical path of cuvette, 1cm

V<sub>s</sub>: Sample volume, 0.2mL

V<sub>sp</sub>: 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°C 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  $\Delta A = A_2 - A_1 = 0.19 - 0.178 = 0.012$ .

$$\text{GBSS activity (U/g mass)} = 43.2 \times \Delta A \div W = 5.184 \text{ U/g mass.}$$

2. Take 0.1g willow and add 1mL extract solution, homogenize in ice bath. centrifugation at 4°C 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  $\Delta A = A_2 - A_1 = 1.919 - 1.915 = 0.004$ , and calculate the enzyme activity according to the sample mass.

$$\text{GBSS activity (U/g mass)} = 43.2 \times \Delta A \div W = 1.728 \text{ U/g mass.}$$

**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