Granule Bound Starch Synthase

Microplate Assay Kit

Cat #: orb545615 (manual)

Detection and Quantification of Granule Bound Starch Synthase Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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INTRODUCTION

In enzymology, a starch synthase (EC 2.4.1.21) is an enzyme that catalyzes the chemical reaction.

ADP-glucose + (1, 4-alpha-D-glucosyl) $_n$ → ADP + (1, 4-alpha-D-glucosyl) $_{n+1}$

Thus, the two substrates of this enzyme are ADP-glucose and a chain of D-glucose residues joined by 1, 4-alpha-glycosidic bonds, whereas its two products are ADP and an elongated chain of glucose residues. Plants use these enzymes in the biosynthesis of starch.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 7	4 °C
Diluent	30 ml x 1	4 °C
Enzyme A	Powder x 1	-20 °C
Enzyme B	Powder x 1	-20 °C
Coenzyme	Powder x 1	-20 °C
Substrate	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
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Note:

Enzyme A: add 5 ml Diluent to dissolve before use.

Enzyme B: add 1 ml Diluent to dissolve before use.

Coenzyme: add 10 ml Diluent to dissolve before use.

Substrate: add 10 ml Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, discard the supernatant, then add 1 ml Assay buffer into the precipitate, mix and keep it on ice for detection.

ASSAY PROCEDURE

Add following reagents into the centrifuge tube:

Reagent	Sample	Standard	Blank
Sample	50 µl	--	--
Substrate	100 µl	--	--
Mix, incubate at 30°C for 30 minutes, put it into boiling water for 2 minutes. Then keep it on ice for cold.			
Enzyme A	50 µl	--	--
Mix, incubate at 30°C for 30 minutes, put it into boiling water for 2 minutes. Then keep it on ice for cold. Centrifuged at 10000g 4 °C for 10 minutes, add the supernatant into the microplate.			
Supernatant	100 µl	--	--
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Coenzyme	90 µl	--	--
Enzyme B	10 µl	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION

Unit Definition: One Unit of Granule Bound Starch Synthase activity is defined as the enzyme produces 1 nmol NADPH per minute.

1. According to the protein concentration of sample

$$GBSS\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S))} / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T1 / T2$$

$$= 53.33 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S))} / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$GBSS\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S))} / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T1 / T2$$

$$= 53.33 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S))} / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$GBSS\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S))} / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T1 / T2$$

$$= 53.33 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S))} / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/ml;

$V_{Standard}$: the volume of standard, 200 μl = 0.2 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Sample}: the volume of sample, 50 / [(50+100+50) /100] = 25 μl = 0.025 ml;

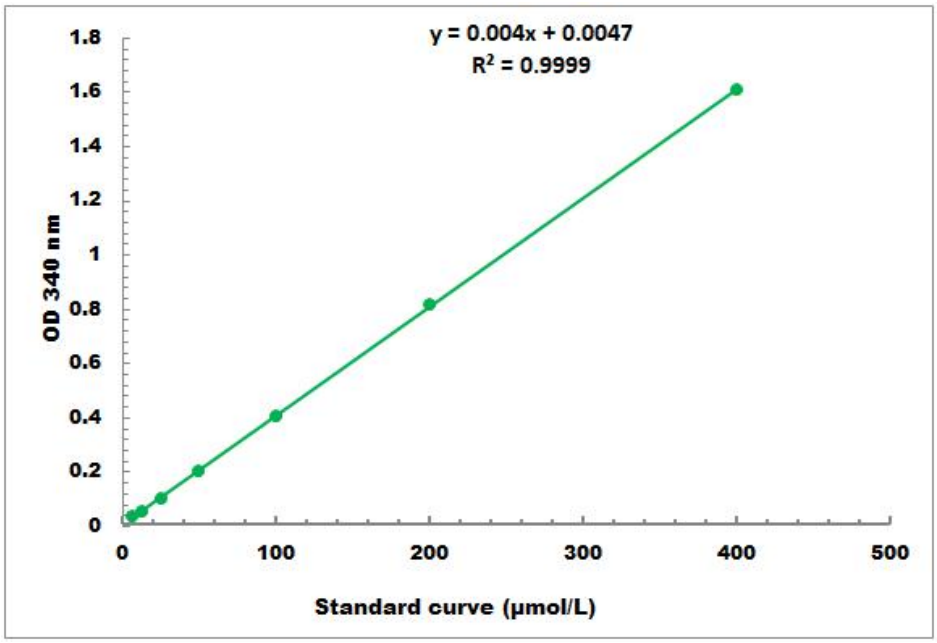
V_{Assay}: the volume of Assay buffer, 1 ml;

T1: the reaction time, 30 minutes.

T2: the reaction time, 2 minutes.

TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 µmol/L - 400 µmol/L

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