Granule Bound Starch Synthase Microplate Assay Kit

Catalog # AS0120

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION

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

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

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.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 7	4 °C
Diluent	30 mlx 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
Technical Manual	1 Manual	

Note:

Enzyme A: add 5 ml diluentto dissolve before use.

Enzyme B: add 1 ml diluentto dissolve before use.

Coenzyme: add 10 ml diluentto dissolve before use.

Substrate: add 10 ml diluentto dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

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

IV. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 10000g 4°C for 10 minutes, discard the supernatant, thenadd 1 mlAssay bufferinto the precipitate, mix and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents intothe centrifuge tube:

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

Note: if the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time.

VI. CALCULATION

Unit Definition: One Unit ofGranule Bound Starch Synthaseactivity is defined as the enzyme produces 1nmolNADPH per minute.

1. According to the protein concentration of sample

$$GBSS\ (U/mg) = (OD_{Sample(130S)} - OD_{Sample(10S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (V_{Sample} \times C_{Protein}) / T1 / T2 \times 2$$

$$= 35.73 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / C_{Protein}$$

2. According to the weight of sample

$$GBSS\ (U/g) = (OD_{Sample(130S)} - OD_{Sample(10S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (W \times V_{Sample} / V_{Assay}) / T1 / T2 \times 2$$

$$= 35.73 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / W$$

3. According to the quantity of cells or bacteria

$$GBSS\ (U/10^4) = (OD_{Sample(130S)} - OD_{Sample(10S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (N \times V_{Sample} / V_{Assay}) / T1 / T2 \times 2$$

$$= 35.73 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / N$$

ε: molar extinction coefficient, 6.22×10^3L/mol/cm;

d: the optical path of 96-Well microplate, 0.6 cm;

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

W: the weight of sample, g;

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

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.05 ml;

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

T1: the reaction time, 30 minutes.

T2: the reaction time, 2 minutes.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

VIII. NOTES