



Granule Bound Starch Synthase Microplate Assay Kit User Manual

Catalog # ASK1130

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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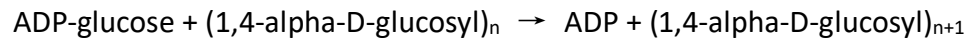
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I. INTRODUCTION

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



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-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
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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.

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

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.



V. ASSAY PROCEDURE

Add following reagents into the centrifuge tube:

Reagent	Sample
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
Coenzyme	90 µl
Enzyme B	10 µl
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.	

VI. 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

$$\begin{aligned} \text{GBSS (U/mg)} &= (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\epsilon \times d) \times V_{\text{Total}} \times 10^9 / (V_{\text{Sample}} \times C_{\text{Protein}}) / \\ & \quad T1 / T2 \times 2 \\ &= 35.73 \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{GBSS (U/g)} &= (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\epsilon \times d) \times V_{\text{Total}} \times 10^9 / (W \times V_{\text{Sample}} / V_{\text{Assay}}) / \\ & \quad T1 / T2 \times 2 \\ &= 35.73 \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{GBSS (U}/10^4) &= (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\epsilon \times d) \times V_{\text{Total}} \times 10^9 / (N \times V_{\text{Sample}} / V_{\text{Assay}}) \\ & \quad / T1 / T2 \times 2 \\ &= 35.73 \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / N \end{aligned}$$

ϵ : molar extinction coefficient, 6.22×10^3 L/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.