



# **Soluble Starch Synthase Microplate Assay Kit User Manual**

**Catalog # CAK1129**

(Version 1.2A)

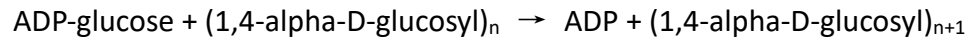
Detection and Quantification of Soluble Starch Synthase Activity in  
Tissue extracts, Cell lysate, Cell culture media and Other biological  
fluids Samples.

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

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## 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 4  | 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  |
| Technical Manual   | 1 Manual   |         |

### 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, multi-channel 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, take the supernatant into a new centrifuge tube and keep it on ice for detection.

## V. ASSAY PROCEDURE

Add following reagents into the centrifuge tube:

| Reagent  | Sample      |
|--|-------------|
| Sample   | 50 $\mu$ l  |
| Substrate  | 100 $\mu$ l |
| 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 $\mu$ l  |
| 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 $\mu$ l |
| Coenzyme   | 90 $\mu$ l  |
| Enzyme B   | 10 $\mu$ l  |
| Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.   |             |

### Note:

- 1) 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.
- 2) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

**Unit Definition:** One Unit of Soluble Starch Synthase activity is defined as the enzyme produces 1  $\mu\text{mol}$  NADPH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{SSS (U/mg)} &= (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\epsilon \times d) \times V_{\text{Total}} \times 10^9 / (V_{\text{Sample}} \times C_{\text{Protein}}) / T1 \\ &\quad / T2 \times 2 \\ &= 0.03573 \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{SSS (U/g)} &= (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\epsilon \times d) \times V_{\text{Total}} \times 10^9 / (W \times V_{\text{Sample}} / V_{\text{Assay}}) / \\ &\quad T1 / T2 \times 2 \\ &= 0.03573 \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{SSS (U/10}^4) &= (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\epsilon \times d) \times V_{\text{Total}} \times 10^9 / (N \times V_{\text{Sample}} / V_{\text{Assay}}) / \\ &\quad T1 / T2 \times 2 \\ &= 0.03573 \times (\text{OD}_{\text{Sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / N \end{aligned}$$

$\epsilon$ : molar extinction coefficient,  $6.22 \times 10^3$  L/mol/cm;

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

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

W: the weight of sample, g;

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

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

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

$V_{\text{Assay}}$ : 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.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## **VIII. NOTES**