# CheKine™ Micro Sucrose Phosphorylase (SP) Activity Assay Kit

Cat #: KTB3120 Size: 48 T/96 T

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|---|---|---|---|
| | **Micro Sucrose Phosphorylase (SP) Activity Assay Kit** | | |
| REF | **Cat #:** KTB3120 | LOT | **Lot #:** Refer to product label |
| | **Applicable sample:** Plant tissues, Fungus | | |
| | **Storage:** Stored at -20℃ for 6 months, protected from light | | |

## Assay Principle

SucrosePhosphorylase (SP, EC2.4.1.7) is mainly present in microorganisms and plants and belongs to the glycosylhydrolase 13 family. It is an enzyme that catalyzes the transfer of glucoside bonds and can catalyze the synthesis of 1-phospho-glucose from sucrose and inorganic phosphate. This enzyme mainly uses sucrose and glucose 1-phosphate as donors, and many substances such as polyhydroxyl sugars, sugar alcohols, phenolic hydroxyl groups, carboxyl groups as receptors to catalyze the synthesis of various glycosides. CheKine™ Micro Sucrose Phosphorylase (SP) Activity Assay Kit can detect plant tissues, fungus samples. In this kit, SP can catalyze sucrose to produce glucose 1-phosphate, which is modified to glucose 6-phosphate under the catalysis of glucose phosphomutase, and reduce $NADP^+$ to generate NADPH under the action of glucose 6-phosphate dehydrogenase, resulting in an increase in the light absorption value of 340nm. The SP activity was reflected by the increase rate of 340nm absorbance.

## Materials Supplied and Storage Conditions

| Kit components | Size | | Storage conditions |
|---|---|---|---|
| | 48 T | 96 T | |
| Extraction Buffer | 50 mL | 100 mL | 4℃ |
| Assay Buffer | 5 mL | 10 mL | 4℃ |
| Reagent Ⅰ | 1 | 1 | 4℃, protected from light |
| Reagent Ⅱ | 0.75 mL | 1.5 mL | 4℃, protected from light |
| Reagent Ⅲ | 0.5 mL | 1 mL | 4℃, protected from light |
| Reagent Ⅳ | 1 | 1 | -20℃, protected from light |
| Reagent Ⅴ | 1 | 1 | -20℃, protected from light |
| Reagent Ⅵ | 1 | 1 | -20℃, protected from light |
| Reagent Ⅶ | 1 | 1 | -20℃, protected from light |

## Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV microplate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, cryogenic centrifuge,1.5 mL EP tube
- Deionized water
- Mortar or homogenizer (for tissue samples)

## Reagent Preparation

**Extraction Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

**Assay Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

**Reagent I:** Prepared before use. Add 2.5 mL deionized water for 48 T and 5 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at 4℃ and protected from light for 2 weeks.

**Reagent II:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

**Reagent III:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

**Reagent IV:** Prepared before use. Add 0.5 mL deionized water for 48 T and 1 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

**Reagent V:** Prepared before use. Add 0.5 mL deionized water for 48 T and 1 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

**Reagent VI:** Prepared before use. Add 1 mL deionized water for 48 T and 2 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 2 weeks after aliquoting to avoid repeated freezing and thawing.

**Reagent VII:** Prepared before use. Add 1 mL deionized water for 48 T and 2 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 2 weeks after aliquoting to avoid repeated freezing and thawing.

## Sample Preparation

**Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.**

1. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Fungus: Collect $5\times10^6$ fungus into the centrifuge tube, wash fungus with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the fungus 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

**Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.**

## Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 96-well UV microplate or microquartz cuvette):

| Reagent | Test Well (μL) | Control Well (μL) |
|---|---|---|
| Assay Buffer | 65 | 85 |
| Reagent Ⅰ | 50 | 50 |
| Reagent Ⅱ | 3 | 3 |
| Reagent Ⅲ | 2 | 2 |
| Reagent Ⅳ | 10 | 10 |
| Reagent Ⅴ | 10 | 10 |
| Reagent Ⅵ | 20 | 20 |
| Reagent Ⅶ | 20 | 20 |
| Mix well, hold at 37℃ for 5 min | | |
| Sample | 20 | 0 |

3. Mix thoroughly, measure the absorbance value $A_1$ at 10 s at 340 nm, and the absorbance value $A_2$ at 130 s at 37℃ for 10 min. The Test Well is marked as $A_{Test}$, the Control Well is marked as $A_{Control}$. Finally calculate $\Delta A=(A_{2Test}-A_{1Test})-(A_{2Control}-A_{1Control})$.

**Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.05, increase the sample quantity appropriately. If A is greater than 1 or ΔA is greater than 0.6, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. If ΔA is negative, the sample does not contain SP or is degraded.**

## Data Analysis

**Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.**

Calculation of SP activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: At 37℃, pH6.8, the production of 1 nmol of NAPDH per milligram of protein per min was defined as one unit of enzyme activity.

SP (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T$**$=1608\times\Delta A\div Cpr$**

(2) Calculated by fresh weight of samples

Active unit definition: At 37℃, pH6.8, the production of 1 nmol of NAPDH per gram tissue per min was defined as one unit of enzyme activity.

SP (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**$=1608\times\Delta A\div W$**

(3) Calculated by fungus number

Active unit definition: At 37℃, pH6.8, the production of 1 nmol of NAPDH per $10^4$ fungus min was defined as one unit of enzyme activity.

SP ($U/10^4$) = $[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(n\times V_{Sample}\div V_{Total\ sample})\div T$**$=1608\times\Delta A\div n$**

$V_{Total}$: total reaction volume, $2\times10^{-4}$ L; ε: NADPH molar extinction coefficient, $6.22\times10^3$ L/mol /cm; d: the light path of the 96-well UV plate, 0.5 cm; $V_{Sample}$: sample volume added, 0.02 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 2 min; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; n: Total number of fungus, calculated in units of ten thousand.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

## Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

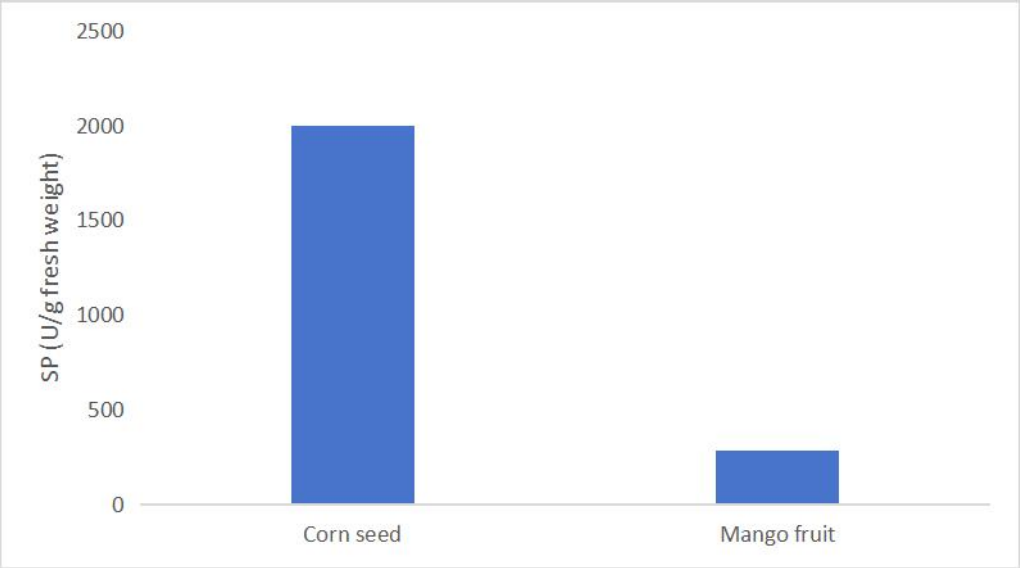


Figure 1. Determination SP activity in corn seed and mango fruit by this assay kit.

## Recommended Products

| Catalog No. | Product Name |
|---|---|
| KTB3110 | CheKine™ Micro Sucrose Synthetase (SS) Activity Assay Kit |
| KTB1560 | CheKine™ Micro Alcohol Acyltransferase (AAT) Activity Assay Kit |
| KTB1270 | CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit |

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.