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CheKine™ Micro Triose-Phosphate Isomerase (TPI) Activity Assay Kit

Cat #: KTB1128 Size: 48 T/96 T

	Micro Triose-Phosphate Isomerase (TPI) Activity Assay Kit		
REF	**Cat #:** KTB1128	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Triose-phosphateisomerase (TPI) in plant chloroplasts is an important enzyme involved in calvin cycle in photosynthesis. Acting on the transformation between glyceraldehyde phosphate and dihydroxyacetone phosphate, dihydroxyacetone phosphate can quickly penetrate the chloroplast envelope and enter the cytoplasm, where it is gradually transformed into sucrose. CheKine™ Micro Triose-Phosphate Isomerase (TPI) Activity Assay Kit can detect plant tissues In this kit, TPI converts dihydroxyacetone phosphate into glyceraldehyde-3-phosphate, and glyceraldehyde-3-phosphate and NAD react with glyceraldehyde-3-phosphate dehydrogenase to generate glyceric acid-3-phosphate and NADH. The absorbance change at 340 nm reflects the activity of TPI.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	50 mL	100 mL	4℃
Extraction Buffer Ⅱ	50 mL	100 mL	4℃
Reagent Ⅰ	5 mL	10 mL	4℃, protected from light
Reagent Ⅱ	1	1	4℃, protected from light
Reagent Ⅲ	1	1	-20℃, protected from light
Reagent Ⅳ	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 micro quartz cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

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

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

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

Reagent Ⅱ: 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 1 month after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅲ: 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 1 month after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅳ: 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 1 month 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.

Plant Tissues: ① **Extraction of total TPI:** weigh about 0.1 g sample, add 1ml of Extraction Buffer Ⅰ, homogenize in ice bath, and ultrasonically crush for 30 times (power 20% or 200 W, ultrasonic for 3 s, interval 7 s), then centrifuge at 4℃ for 10 min at 8,000 g, and take the supernatant and put it on ice for testing. ② **Separation of TPI cytoplasm and chloroplast:** Weigh about 0.1 g sample, add 1 ml of Extraction Buffer Ⅰ, homogenize 200 g in ice bath, centrifuge at 4℃ for 5 min, discard the precipitate, take 8,000 g of supernatant, centrifuge at 4℃ for 10 min, **take the supernatant to determine the activity of cytoplasmic TPI**, and add 1 ml of Extraction Buffer Ⅱ to the precipitate. After shaking and dissolving, it was crushed by ultrasonic for 30 times (power 20% or 200 W, ultrasonic for 3 s, interval 7 s), then 8,000 g was centrifuged at 4℃ for 10 min, **and the supernatant was taken to determine the TPI activity of chloroplasts**.

Note: It is recommended to determine the total TPI activity and extract the crude enzyme solution according to step ①. If TPI in cytoplasm and chloroplast needs to be determined separately, extract the crude enzyme solution according to step ②.

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. Reagent Ⅰ place at 37℃ incubation for 10 min.
3. Operation table (The following operations are operated in the 96-well UV microplate or micro quartz cuvette **in turn**):

Reagent	Test Well (μL)
Reagent Ⅰ	120
Reagent Ⅱ	20
Reagent Ⅲ	20
Reagent Ⅳ	20

Sample	20

4. Mix thoroughly, measure the absorbance value A_1 at 10 s at 340 nm, and the absorbance value A_2 at 310 s at 37℃ for 5 min. Finally calculate $\Delta A=A_2-A_1$.

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 ΔA is greater than 0.8, 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 TPI 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 TPI activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: The consumption of 1 nmol of NADH per milligram of protein per min was defined as one unit of enzyme activity.

TPI (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T$**$=643.08\times\Delta A\div Cpr$**

(2) Calculated by fresh weight of samples

Active unit definition: The consumption of 1 nmol of NADH per gram tissue per min was defined as one unit of enzyme activity.

TPI (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**$=643.08\times\Delta A\div W$**

V_{Total}: total reaction volume, 0.2 mL; ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol /cm; d: the light path of the 96-well plate, 0.5 cm; V_{Sample}: sample volume added, 0.02 mL; $V_{Total\ sample}$: Extraction Buffer II volume added, 1 mL; T: reaction time, 5 min; Cpr: sample protein concentration, mg/mL; W: weight of sample, g.

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.

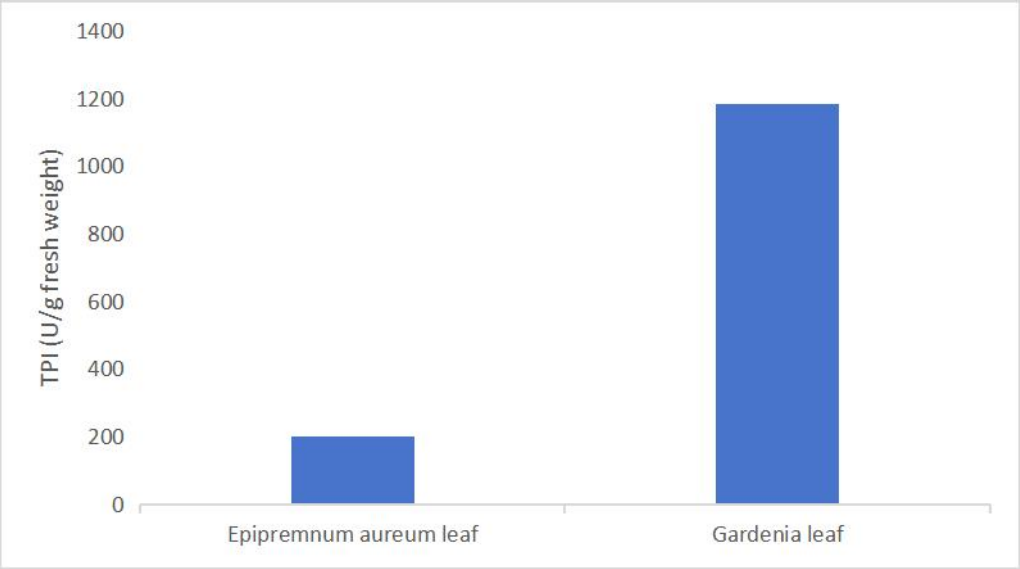

Figure 1. Determination TPI activity in *Epipremnum aureum* leaf and gardenia leaf by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB3030	CheKine™ Micro Alcohol Dehydrogenase (ADH) 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.