

Triose phosphate isomerase (TPI) activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: BC2265

Size: 100T/96S

Components:

Extract I: Liquid 110mL×1. Store at 2-8°C.

Extract II: Liquid 110mL×1. Store at 2-8°C.

Reagent I: Liquid 13mL×1. Store at 2-8°C.

Reagent II: Powder×2. Store at -20°C. Add 1.1 mL of distilled water to each Reagent II before use. It could be stored at -20°C for four weeks after dispensing to avoid repeated freezing and thawing.

Reagent III: Powder×2. Store at -20°C. Add 1.1 mL of distilled water to each Reagent III before use. It could be stored at -20°C for four weeks after dispensing to avoid repeated freezing and thawing.

Reagent IV: Powder×2. Store at -20°C. Provide a blank bottle. Add 2.5 mL of distilled water to each Reagent IV before use. It could be stored at -20°C for two weeks after dispensing to avoid repeated freezing and thawing.

Product Description:

Triose phosphate isomerase in plant chloroplasts is an important enzyme involved in the calvin cycle in photosynthesis. Triose phosphate isomerase acts on the conversion between glyceraldehyde phosphate and dihydroxyacetone phosphate. Dihydroxyacetone phosphate can quickly penetrate the chloroplast envelope into the cytoplasm, where it is gradually converted into sucrose.

Triose phosphate isomerase converts dihydroxyacetone phosphate to glyceraldehyde 3-phosphate. Glyceraldehyde 3-phosphate and NAD generate 3-phosphoglycerate and NADH under the action of glyceraldehyde 3-phosphate dehydrogenase. The change in absorbance at 340nm reflects the activity of triose phosphate isomerase.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/Microplate reader, balance, water-bath, centrifuge, transferpettor, micro quartz cuvette/96 well UV plate, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation:

1. Extraction of total triose phosphate isomerase:

Take about 0.1g, add 1mL Extract I, ice-bath homogenize and ultrasonically break (ice-bath, power 200w, work time 3s, interval 7s, for 1 min); Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials. Take the supernatant and place it on ice for testing.

2. Separation of Triose Phosphate Isomerase in Cytoplasm and Chloroplast

- 1) According to the plant tissue mass (g): Extract I volume (mL) at a ratio of 1:5-10 (it is recommended to take about 0.1g, add 1mL Extract I), homogenize in an ice bath and centrifuge at $200 \times g$ for 5 minutes at 4°C , discard the precipitate,
- 2) Take the supernatant at 4°C , centrifuge at $8000g$ for 10min, take the supernatant to determine the cytosolic triose phosphate isomerase activity.
- 3) Take the precipitate and add 1mL Extract II. After shaking and dissolving, ultrasonic disintegration (ice bath, power 200w, work time 3 seconds, interval 7 seconds, for 1min); then centrifuge at $8000 \times g$ for 10 minutes at 4°C take the supernatant for determination of triose phosphate isomerase activity in chloroplast.

It is recommended to determine the total triose phosphate isomerase enzyme activity and extract the crude enzyme solution according to **step 1**. If it is necessary to determine the triose phosphate isomerase in the cytoplasm and chloroplast separately, follow the operation of **step 2** to extract the enzyme solution.

II. Determination:

1. Preheat ultraviolet spectrophotometer/Microplate reader for 30 minutes, adjust the wavelength to 340nm, and set spectrophotometer counter to zero with distilled water.
2. Preheat Reagent I at 37°C for 15min
3. Test Tube: Take micro quartz cuvette/96 well UV flat-bottom plate, add $120\mu\text{L}$ Reagent I, $20\mu\text{L}$ Reagent II, $20\mu\text{L}$ Reagent III, $20\mu\text{L}$ Reagent IV, $20\mu\text{L}$ Crude Enzyme Solution, mix well. Record the initial absorbance A_1 at the wavelength of 340 nm for 20 seconds. Incubate it at 37°C for 5min and record the absorbance A_2 at the wavelength of 340nm, $\Delta A = A_2 - A_1$.

III. Calculation:

A. micro quartz cuvette

1. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of generate 1 nmol of NADH per milligram of tissue protein per hour.

$$\text{TPI activity (U/mg prot)} = (\Delta A \div \varepsilon \div d) \times V_{rv} \times 10^9 \div V_s \div C_{pr} \div T \times F = 321.54 \times \Delta A \div C_{pr} \times F$$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of generate 1 nmol of NADH per gram of tissue per hour.

$$\text{TPI activity (U/g weight)} = (\Delta A \div \varepsilon \div d) \times V_{rv} \times 10^9 \div V_s \times V_e \div W \div T \times F = 321.54 \times \Delta A \div W \times F$$

ε : NADH molar extinction coefficient, $6.22 \times 10^3 \text{ L} / \text{mol} / \text{cm}$;

d : Cuvette light path, 1cm;

10^9 : Unit conversion factor, $1 \text{ mol} = 10^9 \text{ nmol}$;

V_{rv} : Total reaction volume, $2.0 \times 10^{-4} \text{ L}$;

V_s : Sample volume, 0.02mL;

V_e : Extract I or Extract II volume, 1mL;

W: Sample weight, g;

Cpr: Sample protein concentration, mg/mL;

T: Reaction time, 5 min;

F: Dilution times.

B. 96 well UV flat-bottom plate:

Modify d-1 cm in the above formula to d-0.6 cm (light path of the 96-well plate) for calculation.

Experimental example:

1. Take 0.11g *Epipremnum aureum* leaf, add 1 mL of Extraction I, grind the homogenate with ice bath and ultrasonically break. Then operate according to the determination steps (use cuvette), calculate $\Delta A = A_2 - A_1 = 0.1641 - 0.1265 = 0.0376$, and calculate the enzyme activity according to the sample mass:

TPI activity (U/g weight) = $321.54 \times 0.0376 \div 0.11 = 109.91$ U/g weight.

2. Take 0.1033g *Yulania denudata* leaf, add 1 mL of Extraction I, grind the homogenate with ice bath and ultrasonically break. Then operate according to the determination steps (use cuvette), calculate $\Delta A = A_2 - A_1 = 1.0696 - 1.0256 = 0.044$, and calculate the enzyme activity according to the sample mass:

TPI activity (U/g weight) = $321.54 \times 0.044 \div 0.1033 = 136.96$ U/g weight.

Related Products:

BC0530/BC0535	Phosphofructokinase (PFK) Activity Assay Kit
BC0540/BC0545	Pyruvate Kinase (PK) Activity Assay Kit
BC0740/BC0745	Hexokinase (HK) Activity Assay Kit
BC2190/BC2195	Phosphoenolpyruvate carboxylase (PEPC) Activity Assay Kit
BC2210/BC2215	Glyceraldehyde-3-phosphate Dehydrogenase(GAPDH) Activity Assay

Kit