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Triose phosphate isomerase（TPI）activity Assay Kit

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

Operation Equipment: Ultraviolet Spectrophotometer

Cat No: AK0786-50T-48S

Size: 50T/48S

Components:

Extract I: Liquid 60mL×1. Store at 2-8℃.

Extract II: Liquid 60mL×1. Store at 2-8℃.

Reagent I: Liquid 35mL×1. Store at 2-8℃.

Reagent II: Powder×2. Store at -20℃. Add 3 mL of distilled water to each Reagent II before use. It could be stored at -20℃ for four weeks after dispensing to avoid repeated freezing and thawing.

Reagent III: Powder×2. Store at -20℃. Add 3 mL of distilled water to each Reagent III before use. It could be stored at -20℃ for four weeks after dispensing to avoid repeated freezing and thawing.

Reagent IV: Powder×3. Store at -20℃. Provide a blank bottle. Add 2.5 mL of distilled water to each Reagent IV before use. It could be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.

Product Description:

Triose phosphate isomerase is an important glycolytic enzyme, which is widely present in animals, plants and microorganisms. Triose phosphate isomerase participates in glucose metabolism in the body. Triose phosphate isomerase plays an important role in glycolysis, gluconeogenesis, fatty acid biosynthesis and pentose phosphate metabolism.

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, balance, water-bath, centrifuge, transferpettor, 1mL quartz cuvette, 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℃ 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 ×g for 5 minutes at 4℃, discard the precipitate,
2) Take the supernatant at 4℃, 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 ×g for 10 minutes at 4℃ 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 for 30 minutes, adjust the wavelength to 340nm, and set counter to zero with distilled water.
2. Preheat Reagent I at 37℃ for 15min
3. Test Tube: Take 1mL quartz cuvette, add 600μL Reagent I, 100μL Reagent II, 100μL Reagent III, 100μL Reagent IV, 100μL Crude Enzyme Solution, mix well. Record the initial absorbance A1 at the wavelength of 340 nm for 20 seconds. Incubate it at at 37℃ for 5min and record the absorbance A2 at the wavelength of 340nm, ΔA=A2-A1.

III. Calculation:

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.

TPI activity（U/mg prot）= (ΔA÷ε÷d)×Vrv×10^9÷Vs÷Cpr÷T×F=321.54×ΔA÷Cpr×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.

TPI activity（U/g weight）=(ΔA÷ε÷d)×Vrv×10^9÷Vs×Ve÷W÷T×F=321.54×ΔA÷W×F

ε: NADH molar extinction coefficient, 6.22×10^3 L / mol /cm；

d: Cuvette light path, 1cm；

10^9: Unit conversion factor, 1 mol=10^9 nmol;

Vrv: Total reaction volume, 2.0×10^{-4}L；

Vs: Sample volume, 0.02mL；

Ve: 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.

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, calculate ΔA = A2-A1 = 0.172-0.138=0.034, and calculate the enzyme activity according to the sample mass:
   TPI activity（U/g weight）=321.54×0.034÷0.11=99.39 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, calculate ΔA = A2-A1 = 1.101-1.056=0.045, and calculate the enzyme activity according to the sample mass:
   TPI activity（U/g weight）=321.54×0.045÷0.1033=140.07 U/g weight.