Acetolactate synthase (ALS)Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Microplate Reader/ Spectrophotometer

Cat No: BC5525

Size: 100T/48S

Components:

Reagent I A: Powder×1. Store at -20℃.

Reagent I B: Powder×1. Store at -20℃.

Reagent I C: Liquid 120 mL×1. Store at 2-8℃. Dissolve Reagent I A and Reagent I B in Reagent I C before use. Store the unused reagents in separate packages at -20℃ for up to 4 weeks, avoiding repeated freezing and thawing.

Reagent II: Liquid 30mL×1. Store at 2-8℃.

Reagent III A: Powder×1. Store at -20℃.

Reagent III B: Powder×1. Store at -20℃.

Reagent III C: Liquid 60 mL×1. Store at 2-8℃. Dissolve Reagent III A and Reagent III B in Reagent III C before use. Store the unused reagents in separate packages at -20℃ for up to 4 weeks, avoiding repeated freezing and thawing.

Reagent IV: Liquid 3mL×1. Store at 2-8℃.

Reagent V: Liquid 12mL×1. Store at 2-8℃.

Reagent VI: Powder×1. Store at 2-8℃. Add 2mL of anhydrous ethanol to dissolve before use. Unused reagent can be stored at -20℃ for 4 weeks.

Reagent VI Diluent: Liquid 15mL×1. Store at 2-8℃.

Reagent VI working solution: before use according to the sample size according to Reagent VI: Reagent VI dilution = 0.1mL: 0.7mL (a total of 0.8mL, 8T) ratio of preparation, ready to use. Use up on the same day.

Standard: Liquid 1mL×1. Store at 2-8℃. 100 μmol/mL acetoin standard solution (10^5 nmol/mL acetoin standard solution).

Preparation of 100nmol/mL standard solution: Take 50 μL of 10^5 nmol/mL acetoin standard solution and mix it with 950 μL of distilled water,（that is5000 nmol/mL standard solution）.Then take 20 μL of 5000 nmol/mL standard solution and mix it with 980 μL of distilled water, (that is 100 nmol/mL standard solution).

Product Description:

Acetolactate Synthase (ALS, EC 4.1.3.18), also known as Acetohydroxyacid synthase (AHAS). ALS is present in plant growth and is a key enzyme in the induction of the biosynthesis of the branched-chain amino acids (Ile, Leu, and Val) during the The key enzyme in the first stage of biosynthesis of branched

chain amino acids (Ile, Leu, Val).

ALS catalyzes the formation of acetolactate from pyruvate, which is decarboxylated under certain conditions to form acetoin, which reacts with creatine and α-naphthol under alkaline conditions to form a red substance with a maximum absorbance value at 525 nm.

Reagents and Equipment Required but Not Provided:

Microplate Reader/spectrophotometer, water bath/incubator, benchtop centrifuge, balance, adjustable pipettes, micro glass cuvettes/96-well plates, mortar and homogenizer, distilled water, ice, anhydrous ethanol and ammonium sulphate.

Procedure:

I. **Sample preparation:**

1. Homogenize the tissue in the ratio of tissue mass (g): reagent volume (mL) 1:2~4 (it is recommended to weigh about 0.5g to 1g of tissue and add the reagent to make sure it is volume to 2mL) in an ice bath. centrifuge at 15000g for 20min at 4℃, take 1mL of the supernatant and transfer it to a new 2mL EP tube.

2. Add about 0.5g of ammonium sulfate to the supernatant taken out, dissolve it thoroughly and let it stand at 4℃ for 2 h. Then centrifuge at 15000g 4℃ for 20min and discard the supernatant. Add 0.5mL of reagent II to the precipitate and fully dissolve on ice for use (if calculated by protein concentration, use this crude enzyme solution to determine protein concentration by itself).

Determination procedure:

1. Preheat microplate reader/spectrophotometer for 30 minutes, adjust the wavelength to 525 nm, spectrophotometer set zero with distilled water.

2. Add samples in 1.5 mLEP tubes as follows .

Reagent name (μL)	Test tube(T)	Control tube(C)	Standard tube(S)	Blank tube(B)
Sample	200	200	-	-
Reagent III	200	200	-	-
Reagent IV	-	20	-	-
React for 1 hour at 37℃ protected from light				
Reagent IV	20	-	-	-
Mix well and react at 60℃ for 15min, remove and centrifuge at 8000g for 5min, and take the supernatant in a new EP tube.				
Supernatant	200	200	-	-
Standard	-	-	200	-
Distilled water	-	-	-	200
Reagent V	100	100	100	100
Reagent VI	100	100	100	100

Mix well, react at 60℃ for 15min, then 200 μL was taken in a micro glass cuvette/96-well plate, and the absorbance value at 525 nm was measured and recorded as A_T, A_C, A_S, A_B。$\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$。（Standard tubes and standard blanks only need to be done 1-2 times）

II. Calculation:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of acetolactic acid per minute every milligram of protein.

ALS activity（U/mg prot）= $C_S\times\Delta A_T\div\Delta A_S\times V_R\div（Cpr\times V_S）\div T\times F= 210\times\Delta A_T\div\Delta A_S\div Cpr\times F$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of acetolactic acid per minute every gram of tissue.

ALS activity（U/g weight）= $C_S\times\Delta A_T\div\Delta A_S\times V_R\div（W\div2\div V_E\times V_S）\div T\times F= 210\times\Delta A_T\div\Delta A_S\div W\times F$

C_S: concentration of standard solution, 100nmol/mL; V_R: volume of the reaction system at 37°C, 0.42mL; V_E: volume of reagent III added, 0.5mL; V_S : volume of the sample added, 0.2mL; T: reaction time at 37°C, 1h; Cpr: concentration of protein, mg/mL; W: weight of the sample, g; 2: 2mL sample homogenate taken 1mL ; F: sample dilution times.

Note:

1. If ΔA_T is greater than 1, the sample can be diluted or the 37℃ reaction time can be shortened; if the absorbance value is measured or ΔA_T is less than 0.01, the sample volume can be increased or the 37℃ reaction time can be extended. Modify the formula synchronously for the final calculation.

Experimental example:

1. Weigh 0.5037g carrot tissue, operate according to the pretreatment steps for the extraction of enzyme solution, operate according to the assay steps, measured with a 96-well plate to calculate $\Delta A_T = A_T - A_C$= 0.308-0.198 = 0.11, $\Delta A_S= A_S- A_B$= 0.671-0.173 = 0.498, bring the formula to calculate:
   ALS activity (U/g weight) = 210 x $\Delta A_T \div \Delta A_S \div$ W x F = 92.090 U/g weight

References:

[1] George, MouradJohn, King. Effect of four classes of herbicides on growth and acetolactate-synthase activity in several variants of Arabidopsis thaliana[J]. Planta, 1992, 188(4):491-497.

[2] Hwang I T , Ko Y K , Kim T J , et al. Structure–Activity Relationships of Acetolactate Synthase Inhibition among New Benzenesulfonylureas in Rice (Oryza sativa) and Barnyardgrass (Echinochloa crus-galli var. oryzicola)[J]. Pesticide Biochemistry & Physiology, 2000, 68(3):166-172.

Related Products:

BC4160/BC4165 Proline Dehydrogenase (ProDH) Activity Assay Kit
BC4140/BC4145 Leucine Arylamidase (LAP) Activity Assay Kit
BC4400/BC4405 Nithine-δ-aminotransferase (δ-OAT) Activity Assay Kit

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