Shikimate Dehydrogenase (SD) 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: BC4185

Size: 100T/96S

Components:

Extract solution: Liquid 100 mL×1, store at 4℃. Contains insoluble matter, shake well before use.;

Reagent I: Liquid 10 mL×1, store at 4℃;

Reagent II: Powder×1, store at 4℃ and protect from light. Add 5 mL distilled water when the solution will be used;

Reagent III: Powder×1, store at -20℃ and protect from light. Add 10 mL distilled water when the solution will be used.

Product Description:

Shikimic acid pathway is an important metabolic pathway in plants and microorganisms. Shikimate dehydrogenase (EC 1.1.1.25) is the key enzyme to catalyze the fourth step of shikimate synthesis and metabolism.

Shikimate dehydrogenase catalyzes the production of NADPH from shikimate and NADP. The SD activity can be expressed by measuring the increasing rate of absorbance value at 340 nm.

Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, water-bath, transferpettor, micro quartz cuvette/96 well flat-bottom UV plate, mortar/homogenizer and distilled water.

Protocol

I. Preparation:

1. Tissue: according to the ratio of mass (g): extract solution volume (mL): 1:5-10 to add the extract. It is suggested that add 1 mL of extract solution to 0.1 g of tissue. Homogenate on ice. Centrifuge at 8000 g 4℃ for 10 min. Take the supernatant on ice for test.

2. Cells: first collect bacteria or cells into the centrifuge tube. Discard the supernatant after centrifugation. According to the number of the cells (10^4): the volume of the extract solution (mL) is 500~1000:1. It is suggested that add 1 mL of extract solution to 5 million of cells. Breaking cells by ultrasonic wave in ice bath (power 300W, ultrasonic 3s, interval 7s, total time 3 min). Centrifuge at 8000 g 4℃ for 10 min. Take the supernatant on ice for test.

3. Liquid: direct detection.

II. Determination procedure:

1. Preheat Spectrophotometer/ microplate reader for 30 minutes, adjust wavelength to 340 nm, set the counter to zero with distilled water.

2. Working solution: according to the volume ratio of reagent I: reagent II: reagent III=7:4:8 to prepare. Mix thoroughly for standby.

3. Operation table: add the following reagents to the micro quartz cuvette/96 well UV plate respectively

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Working solution	190	190
Sample	10	
Distilled water		10
Start timing when adding samples. Mix thoroughly. Measure the absorbance value A1 at 340 nm for 20s and A2 at 5min 20s. $\Delta A_B=A2_B-A1_B$. $\Delta A_T=A2_T-A1_T$. $\Delta A=\Delta A_T-\Delta A_B$. Blank tube just needs to test once or twice.		

III. SD Calculation:

a. Micro quartz cuvette

1) Protein concentration:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol NADPH per minute every mg tissue protein in the reaction system.

SD (U/mg prot) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(Cpr\times V_{SA})\div T\times 10^9= 643\times\Delta A\div Cpr$

2) Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol NADPH per minute every gram tissue weight in the reaction system.

SD (U/g weight) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_{SA})\div T\times 10^9=643\times\Delta A\div W$

3) Cells

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol NADPH per minute every 10^4 cells in the reaction system.

SD (U/10^4 cell) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(\text{cell amount}\times V_{SA}\div V_E)\div T\times 10^9=643\times\Delta A\div\text{cells}$

4) Liquid volume

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol NADPH per minute every mL liquid in the reaction system.

SD (U/mL)$=\Delta A\div(\varepsilon\times d)\times V_{RT}\div V_{SA}\div T\times 10^9=643\times\Delta A$

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

d: Light path of cuvette, 1 cm;

V_{RT}: Total reaction volume, 0.0002 L;

V_{SA}: Sample volume, 0.01 mL;

V_E: Extract solution volume, 1 mL;

T: Reaction time, 5 min;

Cpr: Protein concentration, mg/mL;

W: Sample weighr, g;

10^9: Unit conversion coefficient, 1 mol=10^9 nmol.

Cell amount: 10^4 cells as a unit.

b. 96 well flat-bottom plate

The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well UV plate.

Note:

1. The supernatant of sample extraction should be placed on ice for test, and it is recommended to complete the test within 2 hours after sample extraction.
2. The protein concentration of the sample needs to be determined by yourself. Because the extract solution contains a certain concentration of protein (about 1 mg/mL). Therefore, the protein concentration of the extract should be deducted when determining the protein concentration of the sample.
3. When ΔA is greater than 1, it is recommended to dilute the sample before determination. When ΔA is less than 0.01, the reaction time (10 min or 15 min) can be prolonged to determine.
4. Blank tube is the test hole for testing the quality of each reagent component. Under the normal conditions, the change of blank tube shall not exceed 0.01.

Experimental Examples:

1. Take 0.1g of radish leaves, add 1mL extract, and process the sample. Take the supernatant and follow the measurement steps. Measure with micro quartz cuvette and calculate as ΔAt=A2t-A1t=0.2771-0.2433=0.0338, ΔAb=A2b-A1 b=0, ΔA=ΔAt-ΔAb=0.0338, calculated according to sample quality:

SD activity（U/g mass）=643×ΔA÷W=217.334 U/g mass。

Related Products:

BC2030/BC2035 Isocitrate Lyase (ICL) Activity Assay Kit

BC3170/BC3175 Acetokinase (ACK) Activity Assay Kit