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Starch Debranching Enzyme (DBE) 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: AK0095

Size: 100T/48S

Components:

Extract solution: Liquid 100 mL×1, store at 4℃;

Reagent I: Liquid 8 mL×1, store at 4℃;

Reagent II: Powder×1, store at 4℃ . Add 3mL of Reagent I when the solution will be used. The rest of reagent store at 4℃;

Reagent III: Liquid 5 mL×1, store at 4℃;

Reagent IV: Liquid 18 mL×1, store at 4℃;

Standard: Powder×1, 10 mg of glucose. before use, add 1 mL of distilled water to dissolve to prepare 10 mg/mL glucose standard solution. Storage at 4℃ for one week.

Product Description:

Starch debranching enzyme (DBE) can specifically and efficiently break the α- 1,6-glycosidic bonds of amylopectin, and "modify" the structure of starch. Starch debranching enzymes adjust the branch chain. The chain length of the starch side chain plays a key role. The balance of starch branching enzyme and starch debranching enzyme enables amylopectin synthesis.

DBE catalyzes amylopectin to produce reducing sugar, which reacts with 3,5-dinitrosalicylic acid to produce a brown-red substance. The DBE activity can be calculated by measuring its change in absorbance at 540nm.

Required but Not Provided:

Spectrophotometer/Microplate reader, desk centrifuge, water-bath, constant temperature incubator, transferpettor, mortar/homogenizer, micro glass cuvette/ 96 well flat-bottom plate, ice and distilled water, EP tube.

Protocol

I. Preparation:

1. Tissue:

According to the weight of the tissue (g): the volume of the extract solution (mL) is 1: 5 ~ 10. Suggest add 1 mL of extract solution to 0.1 g of tissue. Homogenate on ice. Centrifuge at 15000g 4℃ for 10 minutes. Take the supernatant on ice for test.

2. Cells or bacterial

According to the number of cells or bacteria (10^4): the volume of the extract solution (mL) is 500 ~ 1000: 1. It is suggested to take about 5 million bacteria/cell and add 1mL extract reagent. Bacteria/cell is split by ultrasonication (power 20%, ultrasonic 3s, interval 10s, repeat for 30 times). Centrifuge at 15000 g and 4℃ for 10 minutes. Take the supernatant on ice for test.

3. Serum: detect directly.

II. Determination procedure:

1. Preheat spectrophotometer/ microplate reader for 30 minutes, adjust wavelength to 540 nm, set the counter to zero with distilled water.

2. Dilute 10 mg / mL standard solution with distilled water to 1, 0.8, 0.6, 0.4, 0.2, 0.1 mg/mL standard solution for use.

3. Take a 100 μL of sample in a boiling water bath for 5 minutes (close tightly to prevent water loss), cool to room temperature, centrifuge at 8000g and room temperature for 5 minutes, and take the supernatant for use.

4. Operation table:

Reagent name （μL）	Control tube (Ac)	Test tube (At)	Standard tube (As)	Blank tube (Ab)
Boiled sample	40	-		-
sample	-	40	-	-
Standard	-	-	40	-
Distilled water	-	-	-	40
Reagent I	40	-	40	40
Reagent II	-	40	-	-
Mix well, react accurately for 2 h in 37℃ water bath or constant temperature incubator				
Reagent III	40	40	40	40
Reagent IV	120	120	120	120
Mix well and boil in a boiling water bath for 5 minutes (close tightly to prevent water loss), and immediately cool to room temperature after taking out. The absorbance A at 540 nm was measured and recorded as Ab, At, As, and Ab. Calculate ΔA = At-Ac, ΔAs = As-Ab. control tube is required for each test tube, and the standard tube and blank tube need only be detected once or twice.				

Note: After reacting in a 37 ° C water bath or a constant temperature incubator for 2 h, there may be some precipitate at the bottom of the centrifuge tube. centrifugation is not required, and you can directly enter the next test.

III. DBE Calculation:

1. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis, ΔAs as Y-axis. Take ΔA into the equation to obtain x (nmol/mL).

2. **Protein concentration:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 mg glucose per minutes every milligram tissue protein in the reaction system.

DBE (U/mg prot) = x×Ve÷（Ve ×Cpr）÷T = 0.5x÷Cpr

3. **Sample weight:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 mg glucose per min every gram tissue in the reaction system.

DBE (U/g) = x× Ve÷ W÷T×N = 0.5x÷W

4. **Cells or bacteria:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 mg glucose every 10^4 cells or bacteria in the reaction system per min.

DBE (U/10^4 cell) = x× Ve÷ N ÷T =0.5x÷N

5. **Liquid:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 mg glucose every mL liquid in the reaction system per min.

DBE (U/mL) = x× Vs÷Vs÷T =0.5xb

Ve: volume used in the extraction solution, 1mL;

Vs: the volume of added sample, 0.04mL;

Cpr: sample protein concentration, mg/mL, Protein concentration needs to be determined by yourself;

W: Fresh weight of sample, g;

T: React time, 2h.

N: number of cells (10^4) .

Note:

1. When A is greater than 1.5, it is recommended to dilute the sample with the extraction solution before measuring.

1. It is suggested that the cooling time after boiling water bath should be the same in each experiment.

Experimental examples:

1. Take 0.1 g of rice and add 1 mL of Extract solution for sample processing. After centrifugation, the supernatant was placed on ice for testing. The supernatant was diluted 10 times with the extract, and then the determination procedure was followed. After determination with 96 well flat-bottom plate, calculate ΔA=At-Ac=0.243-0. 111=0. 132. Bring the result into the standard curve y=1.3913x-0. 138, and calculate x=0. 194.The enzyme activity is calculated according to the sample mass.

DBE (U/g)=0.5x÷W×10（dilution times）=9.7 U/g.

Related products:

AK0244/AK0243	β-amylase Activity Assay Kit
AK0413/AK0615	Soluble Starch Synthase(SSS) Activity Assay Kit
AK0364/AK0363	Bound Station amylosynthease Activity Assay Kit
AK0258/AK0257	Starch Branching Enzyme(SBE) Activity Assay Kit