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Basic xylanase (BAX) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: AK0132

Size:100T/48S

Components:

Buffer: 60mL×1, storage at 4℃ .

Reagent 1: 7mL×1, storage at 4℃ .

Reagent 2: 10mL×1, storage at 4℃ .

Standard: Powder×1, storage at 4℃ . 10mg of xylose, add 0.667mL of distilled water to dissolve before use, prepare a 100μmol/ mL standard solution. Dilute 50 times to prepare 2μmol / mL xylose standard solution for use.

Product Description:

Xylanase (EC 3.2.1.8) is mainly produced by microorganisms and can catalyze the hydrolysis of xylan, also known as pentosanase or hemicellulase. It can decompose the cell wall of raw materials and β - glucan in brewing or feed industry. It is widely used in brewing and feed industry to reduce the viscosity of materials, promote the release of effective substances, reduce the non-starch polysaccharides in feeding, and promote the absorption and utilization of nutrients. In general, alkaline xylanase (Bax) is isolated from microorganisms with optimal growth pH of 9- 11.

BAX catalyzes the degradation of xylan into reducing oligosaccharides and monosaccharides in an alkaline environment, and further develops a color reaction with 3,5-dinitrosalicylic acid in a boiling water bath. The color of the reaction solution is proportional to the amount of reducing sugar produced by the enzymatic hydrolysis. The BAX activity can be calculated by measuring the increase rate of the absorbance of the reaction solution at 540nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ microplate reader, constant temperature water bath, adjustable transferpettor, balance, mortar/homogenizer, centrifuge, micro glass cuvette/96 well flat-bottom plate and distilled water.

Sample preparation:

1. Plant and animal tissues: Weigh about 0.1 g of sample, add 1 mL of buffer and fully grind. Centrifuge at 8000rpm and 4℃ for 15 min. Dilute the supernatant with distilled water 10 times for test..
2. Fermentation broth: The fermentation broth is centrifuged at 8000 rpm, 4℃ for 15 min, and the supernatant is taken as a sample to be tested.
3. Enzyme dry powder: Weigh about 1mg, add 1mL of buffer to dissolve, Centrifuge at 8000rpm and 4℃ for 15 min. and dilute with distilled water 10 times for testing.

Procedure:

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

2. Add reagent to a 1.5mL EP tube:

Reagent name （μL）	Control tube (Ac)	Test tube (At)	Blank tube (Ab)	Standard tube (As)
Sample	60	60		
2μmol/mL Standard				60
Distilled water			60	
buffer	90	90	90	90
Reagent 1		60	60	60
Mix well, react in a water bath at 50℃ for 30 min, and inactivate immediately in a boiling water bath for 10 min. (Be careful not to let the lid pop open, so as not to enter the water and change the reaction system)				
Reagent 1	60			
Reagent 2	90	90	90	90

Mix well, develop color for 5 min in a boiling water bath (be careful not to open the lid to prevent water from changing the reaction system). After cooling, absorb 200μL in a 96-well plate or cuvette, and measure the absorbance at 540nm of each tube as soon as possible. Recorded as A measurement, A_C, A_S, A_B . Calculate ΔA = At-Ac, ΔAs = As-Ab.

Calculation:

1. **Fermentation broth:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1μmol reducing sugar per minutes every mL fermentation broth in the reaction system.

BAX (U/mL) = Cs× ΔA÷ ΔAs÷ T=0.067×ΔA÷ ΔAs.

Cs： standard concentration， 2μmoL/mL；

T： reaction time, 30min

2. **Enzyme dry powder:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1μmol reducing sugar per minutes every mg powder in the reaction system.

BAX (U/mg prot) = $10\times Cs\times\Delta A\div\Delta As\times Ve\div W_1\div T = 0.67\times\Delta A\div\Delta As\div W_1$

10： Sample dilution factor；

Cs： standard concentration， 2μmoL/mL；

T： reaction time, 30min

Ve： buffer volume， 1mL；

W_1： enzyme dry powder weight， mg；

3. **Tissue:**

(1) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1μmol reducing sugar per minute every gram tissue in the reaction system.

BAX （U/g） = $10\times Cs\times\Delta A\div\Delta As\times Ve\div W_2\div T = 0.67\times\Delta A\div\Delta As\div W_2$

(2) Protein concentration:

Unit definition: ne unit of enzyme activity is defined as the amount of enzyme that catalyzes the production
of 1μmol reducing sugar per min every mg protein in the reaction system.

BAX（U/mg prot）= 10×Cs×ΔA÷ΔAs×Vs÷(Vs×Cpr)÷T =0.67×ΔA÷ΔAs÷Cpr.

10： Sample dilution factor factor；
Cs： standard concentration， 2μmoL/mL;
T： reaction time, 30min;
Ve： buffer volume， 1mL;
W_2： sample weight， g;
Cpr： protein concentration， mg/mL;
Vs： sample volume， 0.06mL.

Precautions:

The change in absorbance should be controlled between 0.01 and 1.2, otherwise increase the sample volume or dilute the sample. Note that the dilution factor involved in the calculation should be changed accordingly.

Experimental examples:

1. Take 0.1 g of leaves and add 1 mL of Buffer for sample processing. Take the supernatant and dilute ten times with distilled water and follow the measurement procedure. After determination with 96 well flat-bottom plate, calculate ΔA = At-Ac=0.584-0.428=0. 156, ΔAs = As-Ab=0.470-0. 127=0.343. The enzyme activity is calculated according to the sample mass.
   BAX（U/g）=0.67×ΔA÷ΔAs÷W_2=3.047 U/g.
2. Take the yeast by centrifugation and take the supernatant and follow the determination procedure. After determination with 96 well flat-bottom plate, calculate ΔA = At-Ac=0.599-0.543=0.056, ΔAs = As-Ab=0.470-0. 127=0.343. The enzyme activity is calculated according to the sample mass.
   BAX (U/mL）=0.067×ΔA÷ ΔAs =0.011 U/mL.

Related products:

AK0199/AK0198 Acidic Xylanase Activity Assay Kit
AK0201/AK0200 Neutral Xylanase Activity Assay Kit
AK0197/AK0196 β-xylosidase Activity Assay Kit