Alkaline xylanase(BAX) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: BC3610

Size: 50T/24S

Components:

Buffer Fluid: Liquid 50 mL×1, store at 2-8℃.

Reagent I: Liquid 15 mL×1, store at 2-8℃.

Reagent II: Liquid 25 mL×1, store at 2-8℃.

Standard: Powder×1, 10mg xylose. Before use, a standard solution of 100μmol/mL was prepared by adding 667μL distilled water and stored at 2-8℃ for 8 weeks.

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 break down the cell walls and β-glucan of raw materials in brewing or feed industries, which reduces the viscosity of materials in brewing, promotes the release of effective substances. As well as it can reduce non starch polysaccharides in feed and promoting the absorption and utilization of nutrients. So it is widely used in brewing and feed industry. Alkaline xylanase (BAX) is generally isolated from microorganisms with an optimal growth pH of 9-11.

BAX catalyzes the degradation of xylan into reducing oligosaccharides and monosaccharides in alkaline environments. Under boiling water bath conditions, it further undergoes a color reaction with 3,5-dinitrosalicylic acid, with a characteristic absorption peak at 540nm. The color depth of the reaction solution is directly proportional to the amount of reducing sugars produced by enzymatic hydrolysis. By measuring the rate of increase in absorbance of the reaction solution at 540nm, BAX activity can be calculated.

Required but not provided:

Spectrophotometer, 1mL glass cuvette, balance, desk centrifuge, water bath, mortar/homogenizer, adjustable pipette, ice and distilled water.

Procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to in the literature)

1. Preparation of fermentation solution for cell or microbial samples: the fermentation solution is centrifuged at 8000rpm, at 4℃, for 15min, supernatant is taken and put on ice for testing.

2. Tissue: weigh 0.1g tissue, add 1mL Buffer Fluid, fully grind on ice. 8000g, centrifuge at 4℃ for 15min, take supernatant, put on ice for testing.

3. Dry enzyme powder: weigh 1mg, add 1mL Buffer Fluid, fully dissolve after shaking and put on

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ice for testing.

Note: Samples with high reducing sugar content (such as plant fruits, etc.) can be properly diluted with distilled water before determination.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 540 nm, set the counter to zero with distilled water.
2. Dilution of standard solution: Using distilled water to dilute the Standard into 4, 3, 2, 1, 0.5μmol/mL of standard solution before measured.
3. Quasi-dilution table:

Num-ber	Predilution concentration (μmol/mL)	Standard volume (μL)	Volume of distilled water (μL)	Diluted concentration (μmol/mL)
1	100	150	1350	10
2	10	200	300	4
3	10	150	350	3
4	10	100	400	2
5	10	100	900	1
6	1	500	500	0.5

Note: 200μL per tube is required in the experiment.

4. Sample determination (add the following reagents in 1.5 mL EP tube in turn).

Reagent Name (μL)	Control tube (A_C)	Test tube (A_T)	Blank tube (A_B)	Standard tube (A_S)
Supernatant	200	200	-	-
Standard	-	-	-	200
Distilled water	-	-	200	-
Buffer fluid	300	300	300	300
Reagent I	-	200	200	200
Mix well, cap tightly, water bath at 50°C for 30min, immediately boil water bath for 10min to inactivate. (Be careful not to let the lid burst, so as not to water, change the reaction system)				
Reagent I	200	-	-	-
Reagent II	300	300	300	300
Mix well, boiling water bath color development for 5min (be careful not to let the lid burst, so as not to change the reaction system), ice bath cooling as soon as possible to measure the absorption value at 540nm wavelength A_C, A_T, A_S, A_B, calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. Blank tube and standard tube only need to be measured 1-2 times.				

III. Calculation

1. Drawing of standard curve:

The standard curve is established according to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔA_S (y, ΔA_S). According to the standard curve, the ΔA_T (y, ΔA_T) is brought into the formula to calculate the sample concentration (x, μmol/mL).

2. Fermentation fluid BAX activity calculation:

Enzyme activity definition: under the condition of 50℃ and pH 9.0, the amount of enzyme required to decompose xylan to produce 1μmol reducing sugar per milliliter of fermentation solution per minute is the activity unit of an alkaline xylanase.

BAX activity (U/mL volume) = $x \div T \times F = x \div 30 \times F$

3. BAX activity calculation of dry enzyme powder:

Enzyme activity definition: under the condition of 50℃ and pH 9.0, the amount of enzyme required to decompose xylan to produce 1μmol reducing sugar per milligram of enzyme per minute is the activity unit of an alkaline xylanase.

BAX activity (U/mg mass) = $x \times V_T \div W_E \div T \times F = x \div W_E \div 30 \times F$

4. Calculation of BAX activity in the tissue:

(1) Calculated by sample protein concentration:

Enzyme activity definition: Under the condition of 50℃ and pH 9.0, the amount of enzyme required to decompose xylan to produce 1μmol reducing sugar per mg of tissue protein per minute is the activity unit of an alkaline xylanase.

BAX activity (U/mg prot) = $x \times V_S \div (V_S \times Cpr) \div T \times F = x \div Cpr \div 30 \times F$

(2) Calculated by sample quality:

Enzyme activity definition: Under the condition of 50℃ and pH 9.0, the amount of enzyme required to decompose xylan to produce 1μmol reducing sugar per gram of tissue per minute is the activity unit of an alkaline xylanase.

BAX activity (U/g mass) = $x \times V_T \div W_S \div T \times F = x \div W_S \div 30 \times F$

V_T: Add Buffer Fluid volume, 1mL;
V_S: Add sample supernatant volume, 0.2mL;
W_E: Mass of enzyme dry powder, mg;
W_S:Sample mass, g;
Cpr: Protein concentration, mg/mL;
T: Reaction time, 30 minutes;
F: Sample dilution ratio;

Note:

The absorbance change should be controlled between 0.01 and 1.3, otherwise increase the sample size or dilute the sample, and pay attention to changing the dilution multiple in the calculation formula simultaneously.

Experimental example:

1. Take 0.1382g Blueberry, add 1ml Buffer Fluid, grind it on ice. Take the supernatant and dilute it 40 times with distilled water and follow the determination procedure. Measured in cuvette, and calculate $\Delta A_T=A_T-A_C$ =0.660-0.561=0.099. Substituting it into the standard curve y=0.3396x-0.1615(R^2=0.9988), calculate x=0.767, and calculate the BAX activity according to the sample mass:

BAX activity (U/g mass) = x÷W2÷30×F =0.767÷0.1382÷30×40=7.40 U/g mass.

2. Centrifuge the kimchi juice and take the supernatant, then follow the measurement steps. Measured in 96 well plate, and calculate $\Delta A_T=A_T-A_C$=0.711-0.116=0.595. Substituting it into the standard curve y=0.3396x-0.1615(R^2=0.9988), calculate x=2.228, and calculate the BAX activity according to the sample volume:

BAX activity (U/mL volume) = x÷T×F =2.228÷30×2=0.149U/mL.

Related Products:

BC2600/BC2605 Acid xylanase (ACX) Activity Assay Kit
BC2590/BC2595 Neutral Xylanase (NEX) Activity Assay Kit
BC2620/BC2625 β- Xylosidase Activity Assay Kit

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