

Acid xylanase (ACX) Activity Assay Kit

Note: The reagents of this product have changed, please operate in strict accordance with the instructions.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC2605

Size: 100T/48S

Components:

Buffer Fluid: Liquid 90 mL×1, store at 2-8°C.

Reagent I: Liquid 8 mL×1, store at 2-8°C.

Reagent II: Liquid 12 mL×1, store at 2-8°C.

Reagent III: Liquid 5 mL×1, store at 2-8°C.

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°C for 8 weeks.

Product Description:

Xylanase (EC 3.2.1.8), produced mainly by microorganisms, catalyzes the hydrolysis of xylan, also known as pentosanase or hemicellulase, can break down the cell wall of raw materials in the brewing or feed industry as well as beta-glucan, reduce the viscosity of brewing materials, promote the release of active substances, and reduce non-starch polysaccharides in feed. Promote the absorption and utilization of nutrients, so it is widely used in the brewing and feed industry, acid xylan (ACX) is generally isolated from acid-resistant fungi, bacteria and some molds.

ACX can degrade xylan into reducing oligosaccharides and monosaccharides in acidic environment, and further react with 3, 5-dinitrosalicylic acid in boiling water bath. There is a characteristic absorption peak at 540nm, and the color depth of the reaction solution is proportional to the amount of reducing sugar produced by enzymatic hydrolysis. The activity of ACX can be calculated by measuring the increase rate of absorption value of the reaction solution at 540nm.

Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, water-bath/ constant temperature incubator, adjustable pipette, micro glass cuvette/96 well plate, mortar /homogenizer, ice and distilled water.

Protocol

I. Sample preparation

1. Fermentation solution: the fermentation solution is centrifuged at 8000rpm, at 4°C, for 15min, supernatant is taken and placed on ice for testing.
2. Dry enzyme powder: weigh 1mg, add 1mL Buffer Fluid, fully dissolve after shaking and put on ice to be measured.
3. Tissue: Weigh 0.1g tissue, add 1mL Buffer Fluid, fully grind on ice. 8000g, centrifuge at 4°C for 15min,

take supernatant, put on ice to be measured.

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/microplate reader for 30 minutes, adjust wavelength to 540 nm, and zero the spectrophotometer with distilled water.
2. Dilution of standard solution: before the use of distilled water to dilute the standard product into 5, 4, 3, 2, 1, 0.5, 0.25 $\mu\text{mol/mL}$ of standard solution to be measured.
3. Quasi-dilution table:

Number	Predilution concentration ($\mu\text{mol/mL}$)	Standard volume (μL)	Volume of distilled water (μL)	Diluted concentration ($\mu\text{mol/mL}$)
1	100	100	900	10
2	10	100	100	5
3	10	80	120	4
4	10	60	140	3
5	10	40	160	2
6	10	40	360	1
7	1	100	100	0.5
8	0.5	100	100	0.25

Note: 60 μL per tube is required in the experiment.

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

Reagent Name (μL)	Contrast tube (A_C)	Test tube (A_T)	Blank tube (A_B)	Standard tube (A_S)
Sample	60	60	-	-
Standard	-	-	-	60
Buffer fluid	90	90	150	90
Reagent I	-	60	60	60

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	60	-	-	-
Reagent II	90	90	90	90
Reagent III	30	30	30	30

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 tubes and standard tube only need to be done 1-2 times.

II. Calculation

1. Drawing of standard curve:

The standard curve is established according to the concentration of the standard tube (x , $\mu\text{mol/mL}$) and the absorbance ΔA_S (y , ΔA_S). According to the standard curve, the ΔA determination (y , ΔA_T) is

brought into the formula to calculate the sample concentration (x , $\mu\text{mol/mL}$).

2. Fermentation fluid ACX activity calculation:

Enzyme activity definition: at 50°C and pH 4.8, the amount of enzyme required to decompose xylan to produce $1\mu\text{mol}$ reducing sugar per milliliter of fermentation solution per minute is one unit of activity of acid xylanase.

$$\text{ACX activity (U/mL)} = x \div T \times F = x \div 30 \times F$$

3. ACX activity calculation of dry enzyme powder:

Enzyme activity definition: under the condition of 50°C and pH 4.8, the amount of enzyme required to decompose xylan to produce $1\mu\text{mol}$ reducing sugar per milligram of enzyme per minute is one unit of activity of acid xylanase.

$$\text{ACX activity (U/mg)} = x \times V_S \div (V_S \times W_E \div V_E) \div T \times F = x \div W_E \div 30 \times F$$

4. Calculation of NEX activity in the organization:

(1) Calculated by sample protein concentration:

Enzyme activity definition: Under the condition of 50°C and pH 4.8, the amount of enzyme required for decomposing xylan to produce $1\mu\text{mol}$ reducing sugar per mg of hiprotein per minute is one unit of activity of acid xylanase.

$$\text{ACX activity (U/mg prot)} = x \times V_S \div (V_S \times C_{pr}) \div T \times F = x \div C_{pr} \div 30 \times F$$

(2) Calculated by sample quality:

Enzyme activity definition: Under the condition of 50°C and pH 4.8, the amount of enzyme required to decompose xylan to produce $1\mu\text{mol}$ reducing sugar per g tissue per minute is one unit of activity of acid xylanase.

$$\text{ACX activity (U/g mass)} = x \times V_S \div (V_S \times W_S \div V_E) \div T \times F = x \div W_S \div 30 \times F$$

V_S : Add sample volume, 0.2mL;

V_E : Add Buffer Fluid volume, 1mL;

W_E : Mass of enzyme dry powder, mg;

W_S : Sample Mass, g;

T : Reaction time, 30 minutes;

C_{pr} : Protein concentration, mg/mL;

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 0.1079g orange, add 1ml extract, grind it on ice. Take the supernatant and dilute it 20 times with distilled water and follow the determination procedure. Measured in 96 well flat-bottom plate, and calculate $\Delta A_T = A_T - A_C = 0.933 - 0.795 = 0.138$, Bring the standard curve $y = 0.2652x - 0.0355$ ($R^2 = 0.9992$), calculate $x = 0.654$, and calculate the ACX activity according to the sample mass:

$$\text{ACX activity (U/g mass)} = x \div W \div 30 \times F = 0.654 \div 0.1079 \div 30 \times 20 = 4.041 \text{ U/g mass.}$$

2. Take pickle juice, centrifuge, take supernatant, and follow the determination procedure. Measured in 96 well flat-bottom plate, and calculate $\Delta A_T = A_T - A_C = 0.354 - 0.245 = 0.109$, Bring the standard curve $y = 0.2652x - 0.0355$ ($R^2 = 0.9992$), calculate $x = 0.545$, and calculate the ACX activity according to the fermentation fluid:

$$\text{ACX activity (U/ mL)} = x \div T \times F = 0.545 \div 30 \times 1 = 0.018 \text{ U/ mL.}$$

Related Products:

BC2590/BC2595 Neutral xylanase (NEX) Activity Assay Kit

BC3610/BC3615 Alkaline xylanase (BAX) Activity Assay Kit