

## Alkaline xylanase(BAX) Activity Assay Kit

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

**Detection equipment:** Spectrophotometer/Microplate reader

**Cat No:** BC3615

**Size:** 100T/48S

### Components:

**Buffer Fluid:** Liquid 70 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.

**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) 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/Microplate reader, micro glass cuvette/96 well plate, 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°C, 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°C 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

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/microplate reader 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 6, 5, 4, 3, 2, 1  $\mu\text{mol/mL}$  of standard solution before measured.
3. Quasi-dilution table:

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

Note: 60  $\mu\text{L}$  per tube is required in the experiment.

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

Reagent Name ( $\mu\text{L}$ )	Control tube ( $A_C$ )	Test tube ( $A_T$ )	Blank tube ( $A_B$ )	Standard tube ( $A_S$ )
Supernatant	60	60	-	-
Standard	-	-	-	60
Distilled water	-	-	60	-
Buffer fluid	90	90	90	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

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 ( $x$ ,  $\mu\text{mol/mL}$ ) and the absorbance  $\Delta A_S$  ( $y$ ,  $\Delta A_S$ ). According to the standard curve, the  $\Delta A_T$  ( $y$ ,  $\Delta A_T$ ) is brought into the formula to calculate the sample concentration ( $x$ ,  $\mu\text{mol/mL}$ ).

2. Fermentation fluid BAX activity calculation:

Enzyme activity definition: under the condition of 50°C and pH 9.0, the amount of enzyme required to decompose xylan to produce 1  $\mu\text{mol}$  reducing sugar per milliliter of fermentation solution per minute is the activity unit of an alkaline xylanase.

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

3. BAX activity calculation of dry enzyme powder:

Enzyme activity definition: under the condition of 50°C and pH 9.0, the amount of enzyme required to decompose xylan to produce 1  $\mu\text{mol}$  reducing sugar per milligram of enzyme per minute is the activity unit of an alkaline xylanase.

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

4. Calculation of BAX activity in the tissue:

(1) Calculated by sample protein concentration:

Enzyme activity definition: Under the condition of 50°C and pH 9.0, the amount of enzyme required to decompose xylan to produce 1  $\mu\text{mol}$  reducing sugar per mg of tissue protein per minute is the activity unit of an alkaline xylanase.

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

(2) Calculated by sample quality:

Enzyme activity definition: Under the condition of 50°C and pH 9.0, the amount of enzyme required to decompose xylan to produce 1  $\mu\text{mol}$  reducing sugar per gram of tissue per minute is the activity unit of an alkaline xylanase.

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

$V_E$ : the volume of Buffer Fluid added in the pre-treatment, 1mL;

$V_S$ : Added sample supernatant volume, 0.06mL;

$W_E$ : Mass of enzyme dry powder, mg;

$W_S$ : Mass of sample, 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 1.2, 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 0.1179g orange, add 1ml Buffer Fluid, grind it on ice. Take the supernatant and dilute it 10 times with distilled water and follow the determination procedure. Measured in 96 well plate, and calculate  $\Delta A_T = A_T - A_C = 1.194 - 1.040 = 0.154$ . Substituting it into the standard curve  $y = 0.2017x - 0.1171 (R^2 = 0.999)$ , calculate  $x = 1.344$ , and calculate the BAX activity according to the sample mass:

$$\text{BAX activity (U/g mass)} = x \div W_2 \div 30 \times F = 1.344 \div 0.1179 \div 30 \times 10 = 3.80 \text{ U/g mass.}$$

2. Centrifuge the kimchi juice and take the supernatant, then follow the measurement steps. Measured in 96 well plate, and calculate  $\Delta A_T = A_T - A_C = 0.925 - 0.169 = 0.756$ . Substituting it into the standard curve  $y = 0.2017x - 0.1171 (R^2 = 0.999)$ , calculate  $x = 4.329$ , and calculate the BAX activity according to the sample volume:

$$\text{BAX activity (U/mL volume)} = x \div T \times F = 4.329 \div 30 \times 1 = 0.144 \text{ U/mL.}$$

**Related Products:**

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