# Acid xylanase (ACX) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC2600

**Size**:50T/24S

**Components:**

Buffer: 75 mL×1, storage at 4℃.

Reagent 1: 10 mL×1, storage at 4℃ and protect from light.

Reagent 2: 15 mL×1, storage at 4℃ and protect from light.

Reagent 3: 5 mL×1, storage at 4℃.

Standard: Powder×1, storage at 4℃. 10 mg of xylose, add 0.667 mL of Buffer to dissolve before use, prepare the 100 μmol/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. Acid xylanase (ACX) is generally isolated from acid-resistant fungi, bacteria, and some molds.

ACX can degrade xylan into reducing oligosaccharides and monosaccharides in an acidic 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 ACX activity can be calculated by measuring the increase rate of the absorbance of the reaction solution at 540 nm.

**Reagents and Equipment Required but Not Provided:**

Spectrophotometer, adjustable transferpettor, balance, mortar/homogenizer, centrifuge, 1 mL glass cuvette and distilled water.

**Sample preparation:**

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

at 8000 rpm and 4℃ for 15 min, and the supernatant is taken as a sample to be tested.

**Procedure:**

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 540 nm and set the counter to zero with distilled water.
2. Add reagent to a 1.5 mL EP tube:

| Reagent name（μL） | Control tube (Ac) | Test tube (At) | Blank tube (Ab) | Standard tube (As) |
|---|---|---|---|---|
| Sample | 200 | 200 | | |
| 2μmol/mL Standard | | | | 200 |
| Buffer | 300 | 300 | 500 | 300 |
| Reagent 1 | | 200 | 200 | 200 |
| Accurate reaction time 30min in 50℃ water bath, then Immediately inactivate 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 | 200 | | | |
| Reagent 2 | 300 | 300 | 300 | 300 |
| Reagent 3 | 100 | 100 | 100 | 100 |

Mix well and boil at 100℃ for 5 min (close tightly to prevent water loss). After cooling, measure the absorbance at 540 nm. Calculate ΔA = At-Ac, ΔAs = As-Ab.

**Calculation:**

1. **Fermentation broth:**

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of reducing sugar in the reaction system per minute at 50℃ and pH 4.8 every mL fermentation broth.

ACX (U/mg prot) = Cs× ΔA÷ ΔAs÷ T=0.067×ΔA÷ ΔAs.

2. **Enzyme dry powder:**

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of reducing sugar in the reaction system per minute at 50℃ and pH 4.8 every mg enzyme.

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

3. **Tissue:**

(1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of reducing sugar in the reaction system per minute at 50℃ and pH 6.0 every mg protein.

ACX（U/mg prot）= $10\times Cs\times\Delta A\div\Delta As\times Vs\div(Vs\times Cpr)\div T = 0.67\times\Delta A\div\Delta As\div Cpr$

(2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of reducing sugar in the reaction system per minute at 50℃ and pH 4.8 every g sample.

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

10：Sample dilution factor；

Cs：standard concentration, 2 μmoL/mL；
T： reaction time, 30 min;
Ve：buffer volume, 1 mL；
$W_1$：enzyme dry powder weight, mg；
$W_2$：sample weight, g；
Cpr：protein concentration, mg/mL；
Vs：sample volume, 0.2 mL.

**Note:**

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 example:**

1. Take 0.1g of orange and add 1 mL Buffer solution for homogenate. Take the supernatant and dilute it ten times with buffer solution, and then operate according to the determination steps. Using 96 well plate, the results show that $A_T$ = 0.911, $A_C$= 0.863, $A_S$ = 0.709, $A_B$ = 0.345

ACX activity (U/g mass) = 0.67 × ($A_T$ - $A_C$) ÷ ($A_S$ - $A_B$) ÷$W_2$ = 0.8835 U/g mass.

**Related Products：**

BC2590/BC2595 Neutral Xylanase Activity Assay Kit
BC3610/BC3615 Alkaline Xylanase Activity Assay Kit