Neutral xylanase (NEX) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: BC2595

Size:100T/48S

Components:

Buffer: 60 mL×1, storage at 4℃.

Reagent 1: 5 mL×1, storage at 4℃ and protect from light.

Reagent 2: 8 mL×1, storage at 4℃ and protect from light.

Standard: powder×1, storage at 4℃. 10 mg of xylose, add 0.667 mL of distilled water to dissolve before use, prepare a 100 μmol/ mL standard solution. Dilute 50 times to prepare 2 μmol/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. Therefore, it is widely used in the brewing and feed industry. Neutral xylanase (NEX) is generally isolated from microorganisms with an optimum growth pH of 6-8.

NEX catalyzes the degradation of xylan into reducing oligosaccharides and monosaccharides in a neutral 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 NEX 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/ microplate reader, adjustable transferpettor, balance, mortar/homogenizer, centrifuge, micro glass cuvette/96 well flat-bottom plate and distilled water.

Sample preparation:

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

10 times for testing.

Procedure:

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 540 nm and set the counter to zero with distilled water.
2. Dilute the tissue supernatant/enzyme solution ten times with distilled water and place on ice for testing.
3. 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	50	50		
2μmol/mL Standard				50
Distilled water			50	
buffer	75	75	75	75
Reagent 1		50	50	50
Accurate reaction time 30min in 50℃ water bath, then immediately inactivated by boiling in a water bath for 10 min.				
Reagent 1	50			
Reagent 2	75	75	75	75

Mix well and boil at 100℃ for 5 min (close tightly to prevent water loss). After cooling, Pipette 200 μL into a 96 well flat-bottom plate, 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 6.0 every mL fermentation broth.

BAX (U/mg prot) = $Cs\times \Delta A\div \Delta As\div T=0.13\times\Delta A\div \Delta As$.

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 6.0 every mg enzyme.

BAX (U/mg) = $10\times Cs\times\Delta A\div\Delta As\times Ve\div W_1\div T =1.33\times\Delta A\div\Delta As\div W_1$

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.

BAX (U/mg prot)= $10\times Cs\times\Delta A\div\Delta As\times Vs\div(Vs\times Cpr)\div T =1.33\times\Delta A\div\Delta As\div Cpr$

(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 6.0 every g sample.

BAX (U/g weight)= $10\times Cs\times\Delta A\div\Delta As\times Ve\div W_2\div T=1.33\times\Delta A\div\Delta As\div W_2$

Cs： standard concentration, 4 μmoL/mL；
W_1： enzyme dry powder weight, mg；
10： Sample dilution factor factor；
T： reaction time, 30 min;
Ve： buffer volume, 1 mL；
W_2： sample weight, g；
Cpr： protein concentration, mg/mL；
Vs： sample volume, 0.05 mL.

Note:
The change in absorbance should be controlled between 0.01 and 1.5, 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 willow leaves and add 1 mL of buffer solution for homogenization. Take the supernatant and dilute it ten times with distilled water. Operate according to the determination steps. Use 96 well plate to measure and calculate: $\Delta A_T = A_T - A_C = 0.592-0.408 = 0.184$, $\Delta A_S = A_S - A_B = 1.009-0.173 = 0.836$
NEX activity (U/g mass) = $1.33\times\Delta A_T \div A_S \div W = 1.33\times 0.184\div 0.836\div 0.1 = 2.927$ U/g mass.

Related Products：
BC2600/BC2605 G6PDH Activity Assay Kit
BC3610/BC3615 Alkaline Xylanase Activity Assay Kit