Neutral Xylanase (NEX) 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: BC2595

Size: 100T/48S

Components:

Buffer Fluid: Liquid 70 mL×1, store at 2-8℃.

Reagent I: Liquid 7 mL×1, store at 2-8℃.

Reagent II: Liquid 10 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:

Aldehyde dehydrogenase (EC 1.2.1.10) is a kind of aldehyde dehydrogenase. It widely exists in vari 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, neutral xylanase (NEX) is generally isolated from the optimal growth pH of 6-8 microorganisms.

NEX catalyzes the degradation of xylan into reducing oligosaccharides and monosaccharides in a neutral environment, and further reacts with 3, 5-dinitrosalicylic acid in a 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 NEX 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. Preparation of fermentation solution for cell or microbial samples: the fermentation solution is centrifuged at 8000rpm, at 4℃, for 15min, supernatant is taken and placed 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 to be measured.

3. Dry enzyme powder: weigh 1mg, add 1mL Buffer Fluid, fully dissolve after shaking and 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 6, 5, 4, 3, 2, 1μmol/mL of standard solution to be measured.
3. Quasi-dilution table:

Number	Predilution concentration（μmol/mL）	Standard volume（μL）	Volume of distilled water（μL）	Diluted concentration（μmol/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: 50μL 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)	Contrast tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Supernatant	50	50	-	-
Standard	-	-	50	-
Distilled water	-	-	-	50
Buffer fluid	75	75	75	75
Reagent I	-	50	50	50
Mix well, cap tightly, water bath at 50℃ 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	50	-	-	-
Reagent II	75	75	75	75
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). After cooling in an ice bath, 200μL is absorbed into the 96-well plate/ micro glass cuvette, and the absorption value at 540nm wavelength is measured as soon as possible. Respectively recorded as 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, μmol/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, μmol/mL).

2. Fermentation fluid NEX activity calculation:

Enzyme activity definition: at 50℃ and pH 6.0, 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 a neutral xylanase.

NEX activity (U/mL) = x÷T×F= x÷30×F

3. NEX activity calculation of dry enzyme powder:

Enzyme activity definition: under the condition of 50℃ and pH 6.0, the amount of enzyme required to decompose xylan to produce 1μmol reducing sugar per milligram of enzyme per minute is the activity unit of a neutral xylanase.

NEX activity (U/mg) =x×V_S ÷ (V_S ×W_E ÷V_E) ÷T×F =x ÷W_E ÷30×F

4. Calculation of NEX activity in the organization:

(1) Calculated by sample protein concentration:

Enzyme activity definition: Under the condition of 50℃ and pH 6.0, the amount of enzyme required for decomposing xylan to produce 1μmol reducing sugar per mg of hiprotein per minute is the activity unit of a neutral xylanase.

NEX activity (U/mg prot) =x×V_S ÷ (V_S ×Cpr) ÷T×F =x ÷Cpr÷30×F

(2) Calculated by sample quality:

Enzyme activity definition: Under the condition of 50℃ and pH 6.0, the amount of enzyme required to decompose xylan to produce 1μmol reducing sugar per g tissue per minute is the activity unit of a neutral xylanase.

NEX activity (U/g mass) =x ×V_S ÷ (V_S ×W_S÷V_E) ÷T×F =x ÷W_S÷30×F

V_S: Add sample volume, 0.05mL;
V_E: Add Buffer Fluid volume, 1mL;
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 1.5, 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 0.1090g orange, add 1ml extract, grind it on ice. Take the supernatant and dilute it 10 times with distilled water and follow the determination procedure. Measured in 96 well flat-bottom plate, and calculate $\Delta A_T=A_T-A_C=0.802-0.739=0.063$, Bring the standard curve y=0.1859x-0.1003 ($R^2=0.9968$), calculate x=0.8784, and calculate the NEX activity according to the sample mass:

NEX activity (U/g mass) = x÷W÷30×F =0.8784÷0.1090÷30×10=2.6862 U/g mass.

2. Take pickle juice, centrifuge, take supernatant, and follow the determination procedure. Measured in 96 well flat-bottom plate, and calculate $\Delta A_T=A_T-A_C=0.782-0.167=0.615$, Bring the standard curve y=0.1859x-0.1003 ($R^2=0.9968$), calculate x=3.848, and calculate the NEX activity according to the sample mass:

NEX activity (U/ mL) = x÷T×F =3.848÷30×1=0.1283 U/ mL.

Related Products:

BC2600/BC2605	Acid xylanase (ACX) Activity Assay Kit
BC3610/BC3615	Alkaline xylanase (BAX) Activity Assay Kit

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