Soil Acid Xylanase (S-ACX) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: BC5720

Size: 50T/24S

Components:

Buffer Fluid: Liquid 40 mL×1, store at 2-8℃.

Reagent I: Liquid 20 mL×1, store at 2-8℃.

Reagent II: Liquid 17 mL×1, store at 2-8℃.

Standard: Powder×1, 10mg xylose.

Standard dilution solution: Before use, prepare according to the ratio of buffer solution: Reagent Ⅰ=4.8mL: 2.4mL (7.2mL, about one standard curve dilution amount), and use it immediately.

Standard: Before use, a standard solution of 100μmol/mL was prepared by adding 667μL standard dilution solution and stored at 2-8℃ for 8 weeks.

Product Description:

Soil acid xylanase (S-ACX), also known as soil acidic hemicellulase, is mainly isolated from microorganisms with an optimal growth pH of 4-5.

In acidic environments, S-ACX catalyzes the degradation of xylan into reducing oligosaccharides and monosaccharides. 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, S-ACX activity can be calculated.

Required but not provided:

Spectrophotometer, 1mL glass cuvette, balance, desk centrifuge, water bath, 30-50 mesh sieve, 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)

Fresh soil samples are naturally air dried or air dried in a 37 ℃ oven, and sieved through a 30-50 mesh sieve.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 540 nm, set the counter to zero with distilled water.

2. Dilution of standard solution: Using **standard dilution solution** to dilute the Standard into 1.5,

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1.2, 1, 0.8, 0.6, 0.4μmol/mL of standard solution before measured.

3. Quasi-dilution table:

Num-ber	Predilution concentration（μmol/mL）	Standard volume（μL）	Volume of **standard dilution solution**（μL）	Diluted concentration（μmol/mL）
1	100	100	900	10
2	10	150	850	1.5
3	10	120	880	1.2
4	10	150	1350	1
5	1	400	100	0.8
6	1	300	200	0.6
7	1	200	300	0.4

Note: 300μL 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)	Control tube (A_C)	Test tube (A_T)	Blank tube (A_B)	Standard tube (A_S)
Sample	0.1g	0.1g	-	-
Buffer Fluid	400	400	-	-
Reagent I	-	200	-	-
Mix well and place in a 50℃ water bath for 2 hours. Immediately after, heat in a boiling water bath for 10 minutes to deactivate (be careful not to let the lid burst open, to prevent water from entering and altering the reaction system). Cool to room temperature.			-	-
Reagent I	200	-	-	-
At room temperature, centrifuge at 12000g for 10 minutes, and take the supernatant			-	-
Supernatant	300	300	-	-
standard dilution solution	-	-	300	-
Standard	-	-	-	300
Reagent II	200	200	200	200
Mix well, accurately color in a boiling water bath for 5 minutes (be careful not to let the lid burst open to avoid water entering and altering the reaction system), cool to room temperature.				
Distilled water	500	500	500	500

After thorough mixing, centrifuge at room temperature for 5 minutes at 12,000g. Take the supernatant and measure the absorbance at 540nm in a 1mL glass cuvette. Record the absorbance values as A_C, A_T, A_B, A_S, calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. The blank tube and standard curve only need to be measured 1-2 times. Each test tube should have a corresponding control tube.

III. 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_T (y, ΔA_T) is brought into the formula to calculate the sample concentration (x, μmol/mL).

2. Calculation of soil S-ACX activity:

Enzyme activity definition: An enzyme activity unit of an acid xylanase is defined as the amount of enzyme required to produce 1 μmol of reducing sugar per gram of soil per hour by decomposing xylan at 50℃ and pH 4.8.

S-ACX activity (U/g soil) = $x \times V_{RV} \div W \div T \times F = 0.3 \times x \div W \times F$

V_{RV}: Total reaction volume, 0.6mL;
W: Sample mass, g;
T: Reaction time, 2h;
F: Sample dilution ratio;

Note:

1. If ΔA_T is less than 0.01, the sample size can be appropriately increased or the reaction time can be extended by 50 ℃ before measurement; If the ΔA_T is greater than1.5 or A_T is greater than 1.5, the supernatant can be diluted with distilled water before measurement, and attention should be paid to synchronously modifying the dilution factor in the calculation formula.
2. It is recommended to use a spiral tube to prevent the lid from bursting during the boiling water bath process and change the reaction system.

Experimental example:

1. Take 0.1g of forest soil, perform the first reaction according to the operation table for 2 hours, then dilute the supernatant twice with distilled water and follow the measurement steps. Measure with a 1mL glass cuvette, calculate $\Delta A_T = A_T - A_C = 0.302 - 0.049 = 0.253$, bring in the standard curve $y = 0.5374x - 0.1048$ ($R^2 = 0.9977$), calculate $x = 0.666$, and calculate S-ACX activity based on sample mass:

S-ACX activity (U/g soil) = $0.3 \times x \div W \times F$ = 3.996 U/g soil.

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BC5730/BC5735 Soil Neutral Xylanase（S-NEX）Activity Assay Kit
BC5740/BC5745 Soil Alkaline Xylanase（S-BAX）Activity Assay Kit

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