Soil Neutral Xylanase (S-NEX) Activity Assay Kit

Product code: 67048

Method: Microplate method

Product Introduction

Xylanase (EC 3.2.1.8) is mainly produced by microorganisms. It catalyzes the hydrolysis of xylan and is also known as pentosanase or hemicellulase.

Xylanase can decompose raw-material cell walls in the brewing and feed industries, as well as beta-glucan. It reduces material viscosity during brewing, promotes the release of effective substances, reduces non-starch polysaccharides in feed, and supports the absorption and utilization of nutrients.

Neutral xylanase (NEX) is generally isolated from microorganisms with an optimal growth pH of 6-8. In a neutral environment, NEX catalyzes the degradation of xylan into reducing oligosaccharides and monosaccharides. These products react with 3,5-dinitrosalicylic acid under boiling water bath conditions to produce a color reaction with a characteristic absorption peak at 540 nm.

The color intensity of the reaction solution is proportional to the amount of reducing sugar produced by enzymatic hydrolysis. NEX activity can be calculated by measuring the increase in absorbance at 540 nm.

Product Packing List

Size	Code	Component	Quantity
100T	67048.1	Reagent I	1 bottle
100T	67048.2	Reagent II	1 bottle
100T	67048.3	Buffer	1 bottle
100T	67048.m	Instructions	1 copy

Quality Standards and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Buffer	--	--

Transportation and Storage

Transportation	Transport with ice packs.
Storage	Store at 2-8°C, protected from light. Shelf life: 180 days.

Product Instructions

1. Sample Processing

Air-dry fresh soil samples and pass them through a 30-50 mesh sieve.

2. Assay Procedure

1. Preheat the microplate reader for 30 min and set the wavelength to 540 nm.
2. Prepare the control tube and assay tube according to the table below.

Component	Control Tube	Assay Tube
Soil sample (g)	0.02	0.02
Buffer (μL)	150	100
Reagent I (μL)	--	50

3. Mix thoroughly and react with shaking at 50°C for 30 min.
4. Immediately place the tubes in a 90°C water bath for 10 min.
5. Centrifuge at 8000g and 25°C for 10 min.
6. Take 100 μL of supernatant.
7. Add 100 μL of Reagent II to both the control tube and assay tube.
8. Mix thoroughly and develop color in a 90°C water bath for 5 min.
9. Transfer 180 μL to a 96-well plate and measure the absorbance at 540 nm.

Record the absorbance values as $A_{Control\ Tube}$and $A_{Assay\ Tube}$.

Calculate ΔA as follows: $\Delta A = A_{Assay\ Tube} - A_{Control\ Tube}$.

3. Calculation

Standard curve: $y = 0.8452x + 0.0058$, $R^2 = 0.9989$

Enzyme activity definition: Under the conditions of 50°C and pH 6.0, the amount of enzyme required to decompose xylan in each gram of soil per day to produce 1 μmol reducing sugar is defined as one unit of enzyme activity.

Activity (μmol/d/g soil sample) = $(\Delta A - 0.0058) \div 0.8452 \times V_{total\ reaction} \times 10^3 \div W \div T \div 150 = 56.8 \times (\Delta A - 0.0058) \div W$

$V_{total\ reaction}$	Total reaction volume, 0.15 mL
T	Reaction time, 1/48 d
1000	1 mmol/L = 10^3μmol/L
150	Molecular weight of xylose
W	Soil sample weight, g

Precautions

1. Before formal measurement, select 2-3 samples with large expected differences for pretesting.
2. Required instruments and supplies to be prepared by the user: balance, room-temperature centrifuge, shaker, constant-temperature water bath, microplate reader, and 96-well plate.
3. This 100T kit can test 48 samples.
4. Ensure a 30 min shaking reaction so the enzyme and substrate are in full contact.
5. Use caution with the 90°C water bath to prevent bursting and avoid altering the reaction system.