Oxalate Oxidase Activity Assay Kit

Cat. No. KITG-089 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Oxalate oxidase (OXO) is an oxidoreductase widely present in plants, fungi, and bacteria. Its core function is to catalyze the oxidative decomposition of oxalate (ethanedioic acid), generating carbon dioxide (CO_2) and hydrogen peroxide (H_2O_2). The generated H_2O_2 reacts with a specific chromogenic agent to produce a pink-red product with a maximum absorption peak at 510 nm. OXO activity is calculated from the absorbance change at 510 nm.
Description	Reaction equation: Oxalate + O_2 → $2CO_2$ + H_2O_2 (catalyzed by OXO). Assay principle: Oxalate oxidase catalyzes the oxidation of oxalate to produce hydrogen peroxide. The H_2O_2 then reacts with a specific chromogenic reagent to form a pink-red product. The color intensity is proportional to the amount of H_2O_2 generated and is directly related to OXO activity. Absorbance is measured at 510 nm. Detection method: Colorimetric assay using a microplate reader at 510 nm. Size: 96 assays.
Form	Kit
Applications	Suitable for detecting oxalate oxidase activity in tissue samples, cell samples, bacteria samples, and other biological specimens.
Notes	Materials Required but Not Supplied Microplate reader; 96-well plate; analytical balance; pipettes; mortar; low-temperature centrifuge; and distilled water. Features and Benefits Microplate-based format for high-throughput screening; 96 assays per kit; simple and rapid colorimetric detection; suitable for multiple sample types, including tissue, cells, and bacteria. Technical Notes 1. This procedure is suitable for

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	the majority of routine sample testing. Experimental conditions may be moderately adjusted according to the actual sample condition. 2. It is recommended to select 2 samples for pre-testing before formal experiments to understand the sample characteristics and experimental workflow and to avoid wasting samples and reagents. 3. If the sample amount is increased, the extraction ratio may be adjusted according to cell count (10^4): extraction buffer volume (mL) = 500-1000:1.
Storage	Store all kit components at 4°C. Bring all reagents to room temperature (25°C) before use. Extraction Buffer: Liquid, 100 mL × 1 bottle; store at 4°C. Reagent 1: Liquid, 10 mL × 1 bottle; store at 4°C. Reagent 2: Powder, mg × 1 tube; store at 4°C. Reagent 3: Liquid, 10 mL × 1 bottle; store at 4°C. Standard Tube: Liquid, 1 mL × 1 tube; store at 4°C.
Reconstitution	Reagent 2 Preparation: Before use, gently shake the tube so that the powder falls to the bottom. Add 1.2 mL distilled water to the tube, dissolve, and mix thoroughly. The dissolved reagent may be aliquoted and stored frozen for extended stability.
Reaction conditions	Sample Preparation 1. Tissue samples: Weigh 0.1 g tissue sample. For samples with sufficient moisture, approximately 0.2 g is recommended. Add 1 mL Extraction Buffer, homogenize in an ice bath, centrifuge at 12,000 rpm for 10 min at 4°C, and use the supernatant for detection. 2. Cell or bacteria samples: Collect the cells or bacteria in a centrifuge tube, centrifuge, and discard the supernatant. Add approximately 5 × 10^6 cells or bacteria to 1 mL Extraction Buffer and grind with a mortar. Sonicate in an ice bath at 200 W using 3 s sonication and 10 s intervals, repeated 30 times. Centrifuge at 12,000 rpm for 10 min at 4°C. Collect the supernatant and keep it on ice for detection. 3. If the sample amount is increased, the extraction ratio may be adjusted according to cell count (10^4): Extraction Buffer volume (mL) = 500-1000:1. Instrument Setup Preheat the microplate reader for 30 min and set the wavelength to 510 nm. Reaction Setup Bring all reagents to room

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	temperature (25°C). Add the following reagents to a 96-well plate in order: 50 μL sample, 90 μL Reagent 1, 10 μL Reagent 2, and 80 μL Reagent 3. Mix thoroughly and read the absorbance at 510 nm as A1. Incubate at 37°C for 30 min, then read the absorbance at 510 nm again as A2. Calculate ΔA = A2 - A1. Assay time: Approximately 30 min, excluding sample preparation time.
Unit definition	Standard Curve y = 9.1927x - 0.0272, where x is the H_2O_2 standard amount (μmol) and y is ΔA. The standard curve is obtained from standard determination; the specific preparation method is provided in the accompanying instructions. 1. Calculation Based on Sample Fresh Weight Unit definition: At 37°C, the amount of enzyme that generates 1 nmol H_2O_2 per minute per gram of tissue is defined as one enzyme activity unit (U). OXO (nmol/min/g fresh weight) = [(ΔA + 0.0272) ÷ 9.1927] ÷ (W × V1 ÷ V) × 10^3 ÷ T = 72.52 × (ΔA + 0.0272) ÷ W. 2. Calculation Based on Sample Protein Concentration Unit definition: At 37°C, the amount of enzyme that generates 1 nmol H_2O_2 per minute per milligram of tissue protein is defined as one enzyme activity unit (U). OXO (nmol/min/mg prot) = [(ΔA + 0.0272) ÷ 9.1927] ÷ (V1 × Cpr) × 10^3 ÷ T = 72.52 × (ΔA + 0.0272) ÷ Cpr. 3. Calculation Based on Cell or Bacteria Count Unit definition: At 37°C, the amount of enzyme that generates 1 nmol H_2O_2 per minute per 10^4 cells or bacteria is defined as one enzyme activity unit (U). OXO (nmol/min/10^4 cells) = [(ΔA + 0.0272) ÷ 9.1927] ÷ (500 × V1 ÷ V) × 10^3 ÷ T = 0.145 × (ΔA + 0.0272). Where: V is the volume of Extraction Buffer added, 1 mL; V1 is the volume of sample added, 0.05 mL; T is the reaction time, 30 min; W is the sample weight, g; 500 is the cell or bacteria count expressed as 500 × 10^4, equivalent to 5 × 10^6 cells or bacteria; and Cpr is the sample protein concentration, mg/mL. A BCA protein content assay kit is recommended for determining Cpr.
Usage	For Research Use Only. Not for Use in Diagnostic or Therapeutic Procedures.

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