Pectinase Assay Kit

Cat. No. Kit-2415 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	
Description	Pectinase is an enzyme that breaks down pectin, a polysaccharide found in plant cell walls. Commonly referred to as pectic enzymes, they include pectolyase, pectozyme and polygalacturonase. One of the most studied and widely used commercial pectinases is polygalacturonase. It is useful because pectin is the jelly-like matrix which helps cement plant cells together and in which other cell wall components, such as cellulose fibrils, are embedded. Therefore pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples and sapota. Pectinases have also been used in wine production since the 1960s. The assay is initiated with the enzymatic hydrolysis of the pectin by pectinase. The enzyme catalysed reaction products react with DNS, and can be measured at a colorimetric readout at 540 nm.
Storage	Shipped and store at 4 degree C for 6 months.
Size	100 Assays
Kit Components	96-Well Microplate: 1 plate Assay Buffer: 30 ml x 4, 4 °C Diluent: 10 ml x 1, 4 °C Substrate: Powder x 1, 4 °C Dye Reagent: 10 ml x 1, 4 °C, keep in dark Standard: Powder x 1, 4 °C Plate Adhesive Strips: 3 Strips

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	Technical Manual: 1 Manual Note: Substrate: add 8 ml Diluent to dissolve before use. Standard: add 1 ml Diluent to dissolve before use, the concentration will be 2 mg/ml.
Materials Required but Not Supplied	1. Microplate reader to read absorbance at 540 nm 2. Distilled water 3. Pipettor 4. Pipette tips 5. Mortar 6. Centrifuge 7. Timer 8. Ice
Preparation	SAMPLE PREPARATION 1. For cell and bacteria samples Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection. 2. For tissue samples Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.
Assay Protocol	Add following reagents into the microplate: Reagent Sample Control Standard Blank Substrate 80 µl 80 µl 80 µl 80 µl Put it in the oven, 50 °C for 5 minutes. Sample 20 µl -- -- -- Boiled Sample -- 20 µl -- --

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	Standard -- -- 20 µl -- Distilled water -- -- -- 20 µl Mix, put it in the oven, 50 °C for 30 minutes. Dye Reagent 100 µl 100 µl 100 µl 100 µl Mix, put it in the oven, 95 °C for 10 minutes, record absorbance measured at 540nm.
Analysis	Unit Definition: One unit of Pectinase activity is the enzyme that generates 1 mg of galacturonic acid per hour at 50 °C, pH 3.5. 1. According to the protein concentration of sample Pectinase (U/mg) = (CStandard × VStandard) × (ODSample - ODControl) / (ODStandard - ODBlank) / (VSample × CProtein) / T = 4 × (ODSample - ODControl) / (ODStandard - ODBlank) / CProtein 2. According to the weight of sample Pectinase (U/g) = (CStandard × VStandard) × (ODSample - ODControl) / (ODStandard - ODBlank) / (W × VSample / VAssay) / T = 4 × (ODSample - ODControl) / (ODStandard - ODBlank) / W 3. According to the quantity of cells or bacteria Pectinase (U/10^4) = (CStandard × VStandard) × (ODSample - ODControl) / (ODStandard - ODBlank) / (N × VSample / VAssay) / T = 4 × (ODSample - ODControl) / (ODStandard - ODBlank) / N CProtein: the protein concentration, mg/ml; W: the weight of sample, g; CStandard: the concentration of standard, 2 mg/ml; N: the quantity of cell or bacteria, N × 104 ; VTotal: the total volume of the enzymatic reaction, 0.2 ml; VStandard: the volume of standard, 0.02 ml; VSample: the volume of sample, 0.02 ml; VAssay: the volume of Assay buffer, 1 ml; T: the reaction time, 0.5 h.

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