CREATIVE BIOMART®
Assay Kit

Pectinase Assay Kit

Product Information

Cat

Kit-2415

Cat.No.

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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

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Plate Adhesive Strips: 3 Strips
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	--	--	--

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Boiled Sample -- 20 µl -- --
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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