Pectinase Assay Kit

Pectinase Assay Kit is a detection kit for the quantification of Pectinase activity in tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82041

Package: 96 wells

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INTRODUCTION

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. 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 function of pectinase in brewing is twofold, first it helps break down the plant (typically fruit) material and so helps the extraction of flavours from the mash. Secondly the presence of pectin in finished wine causes a haze or slight cloudiness. Pectinase is used to break this down and so clear the wine.

Pectinases can be extracted from fungi such as *Aspergillus niger.* The fungus produces these enzymes to break down the middle lamella in plants so that it can extract nutrients from the plant tissues and insert fungal hyphae. If pectinase is boiled it is denatured (unfolded) making it harder to connect with the pectin at the active site, and produce as much juice. [Provide by Wikipedia:Pectinase]

PRINCIPLE OF THE ASSAY

This Pectinase Assay Kit is a simple colorimetric assay that measures the amount of Pectinase present in tissue extracts, cell lysate and cell culture supernatants. 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 O.D. 540 nm. The concentration of Pectinase in the samples is then determined by comparing the O.D. 540 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well Microplate	1 plate	RT
Assay Buffer	30 mL x 4 (ready to use)	4°C
Diluent	10 mL (ready to use)	4°C
Substrate (powder)	1 vial	4°C
Dye Reagent	10 mL (ready to use)	4°C (protect from light)
Standards (powder)	1 vial	4°C
Plate sealer	3 ea	RT
Technical Manual	1 Manual	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 540 nm
- Centrifuge
- Oven
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and bacteria samples: Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL of Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of Assay Buffer on ice, centrifuged at 10,000 x g for 10 minutes at 4°C. Take the supernatant into a new centrifuge tube and keep it on ice for detection.

REAGENT PREPARATION

- Substrate: add 8 mL of Diluent to dissolve before use.
- Standards: add 1 mL of Diluent to dissolve before use, the concentration will be 2 mg/mL. Use the 2 mg/mL Standards to prepare a series of standards according to the Table below.

Standard tube	Final standard conc. (mg/mL)	Volume of distilled water (µL)	Volume of 2 mg/mL Standards (µL)
S1	2	0	500
S2	1	250	250 of S1
S3	0.5	250	250 of S2
S4	0.25	250	250 of S3
S5	0.125	250	250 of S4

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Reagent	Sample	Standard	Blank
Substrate	80 µL	80 µL	80 µL
Incubate for **5 minutes** at **50°C** (in the oven).			
Sample	20 µL		
Standard		20 µL	
Distilled water			20 µL
Mix well and incubate for **30 minutes** at **50°C** (in the oven).			
Dye Reagent I	100 µL	100 µL	100 µL
Mix well and incubate for **10 minutes** at **90°C** (in the oven), and then read the absorbance at **O.D. 540 nm**.			

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. 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.

5. According to the protein concentration of sample:

Pectinase (U/mg)

$= \{[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times C_{Protein})\} / T$

$= [4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / C_{Protein}$

6. According to the weight of sample:

Pectinase (U/g)

$= \{[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blankl}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times W / V_{Assay})\} / T$

$= [4 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / W$

7. According to the quantity of cells or bacteria:

Pectinase ($U/10^4$)

$= \{[(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (N \times V_{Sample} / V_{Assay})\} / T$

$= [4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / N$

Note:

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

$C_{Standard}$: the concentration of standard, 2 mg/mL;

$V_{Standard}$: the volume of standard, 0.02 mL;

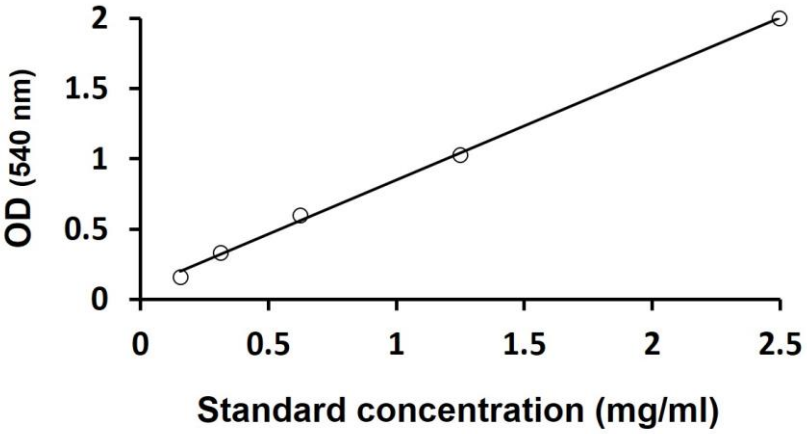
V_{Sample}: the volume of sample, 0.02 mL;

V_{Assay}: the volume of Assay buffer, 1 mL;

N: the quantity of cell or bacteria, $N \times 10^4$.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Pectinase Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.1 mg/mL