QuantiChrom™ Pectinase Assay Kit (DPEC-100)

Quantitative Pectinase Activity Determination

DESCRIPTION

PECTINASE (EC 3.2.1.15) is an enzyme that catalyzes the hydrolysis of pectin, a heteropolysaccharide found primarily in the middle lamella and cell wall of terrestrial plants. During fruit ripening, enzymes, including pectinase break down the middle lamella, separating cells and softening the fruit. Pectinase enzymes are used in the food industry to liquify fruit pulp and clarify fruit juice and wine.

BioAssay Systems' DPEC-100 kit provides a rapid and convenient assay for pectinase enzyme activity, in which pectin the substrate forms a turbid complex with the detection reagent. The turbidity, measured at OD600nm, is proportional to the amount of unhydrolyzed pectin, thus inversely proportional to the pectinase activity.

KEY FEATURES

Sensitive and accurate. Linear detection range from 9.2-100 U/L pectinase activity in a 96-well plate assay.

Simple and high-throughput. Simple and convenient 40 minute "Add-Mix-Measure" type assay. High-throughput format and compatible with laboratory liquid handling systems. No heating required.

Safe. The kit does not use toxic materials. Refer to the previously established DNS method.

Applications

Pectinase activity determination in biological samples (e.g. plant, fungal tissues, juice, bacteria)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Pectin: 8 mL **Reagent:** 15 mL

Calibrator: 500 µL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receipt. Shelf life: 12 months after receipt.

Precautions: Samples should be free of pectin, not turbid, and not intensely colored. Samples with color that absorb at 600 nm may need to be diluted prior to the assay.

PROCEDURES

To ensure identical incubation time, addition of Pectin to samples should be quick. Use of a multi-channel pipettor is recommended. This also applies to the addition of Reagent following incubation. For accurate pectinase activity determination, the assay should be incubated at 25°C.

Sample Preparation: Solid samples can be homogenized in 100mM acetate buffer, pH 4.0. Centrifuge at 14,000 rpm for 5 min and use the supernatant for the assay. Samples must be clear and free of particles.

Reagent Preparation: Allow all kit components to equilibrate to room temperature before the assay. Briefly centrifuge tubes before use.

Calibrator Preparation:

1. Transfer 200 µL of H_2O into two wells of a 96-well plate. Add 20 µL of H_2O to one well (OD_{H2O}) and 20 µL of Calibrator to the other well (OD_{cal}). Pipette up and down to mix the Calibrator.

Reaction Preparation:

1. Transfer 20 µL of each sample into separate wells. Also add 20 µL of H_2O to another well to serve as the blank. Add 60 µL of Pectin to each sample well and the blank well. Tap the plate briefly to mix.
2. Incubate at room temperature for 30 minutes. Add 140 µL of Reagent to each sample and blank well. Tap the plate to mix. Wait 10 minutes and read the optical density at 600 nm.

CALCULATION

Pectinase activity is calculated as follows,

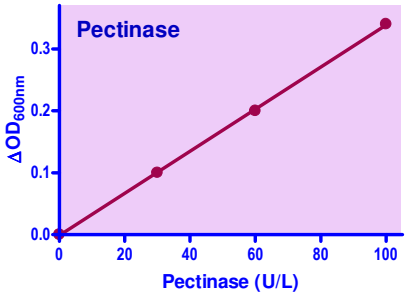
$$\text{Pectinase Activity} = \frac{OD_{Blank} - OD_{Sample}}{OD_{Cal} - OD_{H2O}} \times n \times 100 \text{ (U/L)}$$

OD_{Sample}, OD_{Blank}, OD_{Cal} and OD_{H2O} are the OD600nm values for each sample, Blank, Calibrator and H_2O well, respectively. *n* is the dilution factor if the sample was diluted prior to the assay. *Note: If sample pectinase activity exceeds 100 U/L, dilute samples in water and repeat the assay.*

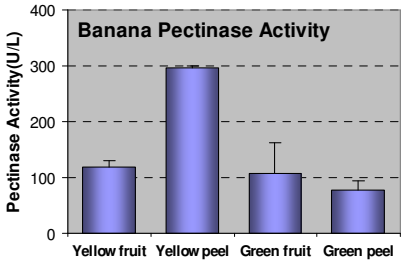
Unit definition: 1 Unit (U) of pectinase will catalyze the hydrolysis of 1 µmole of galacturonic acid from polygalacturonic acid per min at 25°C and pH 4.0.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and a plate reader. Centrifuge if spinning down turbid samples.



Enzyme titration using purified pectinase (Sigma # P4716). Pectinase was diluted in 100mM acetate buffer, pH 4.0 and assayed according to the standard protocol.



Pectinase activity in ripe ("yellow") and green bananas (*Musa acuminata*). Banana fruit and peels were separated and homogenized in 100mM acetate buffer, pH 4.0.

Extracted pectinase in supernatant was assayed in duplicate according to the standard protocol.

LITERATURE

1. Oumer, O. J., & Abate, D. (2018). Screening and Molecular Identification of Pectinase Producing Microbes from Coffee Pulp. Biomed Res Int. 2018: 2961767.
2. Bhadrecha, P., Bala, M., Khasa, Y. P., Arshi, A., Singh, J., & Kumar, M. (2020). Hippophae rhamnoides L. rhizobacteria exhibit diversified cellulase and pectinase activities. Physiology and molecular biology of plants, 26(5), 1075-1085.
3. Jiang, X., Lu, Y., & Liu, S. Q. (2020). Effects of pectinase treatment on the physicochemical and oenological properties of red dragon fruit wine fermented with Torulaspora delbrueckii. Lwt 132, 109929.