Pectinase Assay Kit (BA0221)

Product Datasheet | SKU BA0221

Description

Pectinase (EC 3.2.1.15) is an enzyme that catalyses 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, and pectinase enzymes are used in the food industry to liquefy fruit pulp and clarify fruit juice and wine. In this kit the substrate pectin forms a turbid complex with the detection reagent, and the turbidity measured at 600 nm is proportional to the amount of unhydrolysed pectin, and therefore inversely proportional to pectinase activity.

Specifications

Detection Method	Turbidimetric; read at 600 nm
Detection Range	9.2-100 U/L pectinase activity
Sample Type	Plant, fungal tissues, juice, bacteria
Species Reactivity	All
Assay Time	Approximately 40 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Store all components at -20 degrees C upon receipt.
Shelf Life	12 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Pectin	8 mL	-20 degrees C
Reagent	15 mL	-20 degrees C
Calibrator	500 uL	-20 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0221	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. To ensure identical incubation times, addition of Pectin to samples should be quick and use of a multi-channel pipettor is recommended, which also applies to the addition of Reagent following incubation; for accurate results the assay should be incubated at 25 degrees C.
2. For sample preparation, homogenise solid samples in 100 mM acetate buffer, pH 4.0, centrifuge at 14,000 rpm for 5 minutes and use the supernatant, which must be clear and free of particles.
3. Allow all kit components to equilibrate to room temperature before the assay and briefly centrifuge tubes before use.
4. For the calibrator, transfer 200 microlitres of water into two wells of a 96-well plate, add 20 microlitres of water to one well (OD_H2O) and 20 microlitres of Calibrator to the other well (OD_cal), pipetting up and down to mix the Calibrator.

5. Transfer 20 microlitres of each sample into separate wells and add 20 microlitres of water to another well to serve as the blank, then add 60 microlitres of Pectin to each sample well and the blank well and tap the plate briefly to mix.
6. Incubate at room temperature for 30 minutes, add 140 microlitres 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: Pectinase Activity = [(OD_Blank - OD_Sample) / (OD_Cal - OD_H2O)] x n x 100 (U/L), where OD_Sample, OD_Blank, OD_Cal and OD_H2O are the OD600nm values for each sample, blank, calibrator and water well respectively, and 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 catalyse the hydrolysis of 1 micromole of galacturonic acid from polygalacturonic acid per minute at 25 degrees C and pH 4.0.

Notes

- Samples should be free of pectin, not turbid and not intensely coloured; samples with colour that absorb at 600 nm may need to be diluted prior to the assay.
- For accurate pectinase activity determination, the assay should be incubated at 25 degrees C.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.