Pectin Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6813

Detection and Quantification of Pectin Content in Tissue extractsand Other biological fluids Samples.

For research use only . Not for diagnostic or therapeutic procedures .

1. Introduction

Pectin, any of a group of water-soluble carbohydrate substances that are found in the cell wallsand intercellular tissues of c ertain plants. In the fruits of plants, pectin helps keep the walls of adjacent cells joined together. Immature fruits contain the precursor substance protopectin, which is converted to pectin and becomes more water-soluble as ripening proceeds. At this st age the pectin helps ripening fruits to remain firm and retain their shape. As a fruit becomes overripe, the pectin in it is broken down to simple sugars that are completely water-soluble. As a result, the overripe fruit becomes soft and begins to lose its shape.

Pectic substances consist of an associated group of polysaccharides that are extractable with hot water or with aqueous solutions of dilute acids. The chief sources of commercial pectin are the peels of citrus fruits, and to a lesser extent apple pomace (residue from cider presses). Very small amounts of pectin suffice in the presence of fruit acids and sugar to form a jelly.

Pectin also has several health benefits in humans. Included among these are its ability to reduce low-density lipoprotein (LDL) levels, thereby lowering cholesterol levels, and its ability to slow the passage of food through the intestine, relieving diarrhea. Pectins can also activate cell death pathways in cancer cells, indicating that pectins may play an important role in prev enting certain types of cancer.

Pectin Colorimetric Microplate Assay Kit provides a very sensitive and convenient means to measure pectin content in a variety of samples. In the assay, pectinase reacts with pectin , resulting in the formation of galacturonic acid, which reacts with dye reagent and determined at 540nm, is directly proportional to the pectin content in the sample.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30 mL x 6	RT
Reaction Buffer	30 mL x 1	4 °C
Dye Reagent	10 mL x 1	4 °C, keep in dark
Enzyme	Powderx 1	-20 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Hot air circulation oven

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 10 mmol/ L of standardstock solution, store at 4°C for 3-4 month sor -20°C for 1 year after reconstitution. Then dilute to 3 mmol/ Lstandard top solution by adding 0.3 mL stock solution into 0.7 mL distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve.The concentration of standard curve could be 3/1.5/0.75/0.375/0.188mmol/ L.

Enzyme: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use. Store at 4°C for 1-2 daysor -20°C for 2-3 weeks after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. Sample Preparation

1. For tissue samples Weigh out 0.1 g tissue, homogenize with 1 mL Assay Buffer in the mortar; then transfer all the sample into the microcentrifuge tube; put it into the water bath of 80°C for 30 minutes ; centrifuge at 8, 000g for 1 0minutes , discard the supernatant ; then add 0.5ml Assay Buffer again, mix, put it in water bath of 80°C for 30 minutes, centrifuge at 8, 000g for 10minutes , discard the supernatant; add 200 µL Reaction Buffer into the microcentrifuge tube , mix for detection.

2. For liquid samples Add 10 µL sample and 190 µL Reaction Buffer into the microcentrifuge tube , mix for detection.

6. Assay Procedure

Add following reagents in to the microcentrifuge tube first:

Reagent*	Sample**	Control	Standard	Blank
Reaction Buffer	80 µl	80 µl	--	--
Enzyme	10 µl	10 µl	--	
Sample	10 µl	--	--	--
Distilled water	--	10 µl	--	100 µl
Mix, put it into the water bath, 50 °C for 60 minutes. Centrifuge at 8,000g for 10minutes, add the supernatant into the microplate.				
Standard	--	--	100 µl	--
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, put it into the hot air circulation oven, 90 °Cfor 10 minutes, record absorbance measured at 540nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

$$C = \frac{(OD_{Sample} - OD_{Control}) - Intercept}{Slope} \times \frac{V_{Standard}}{V_{Sample}} \times n \ (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure .

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times C_{Protein} \times V_{Sample}} \ (\mu mol/mg)$$

2.2 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \ (\mu mol/g)$$

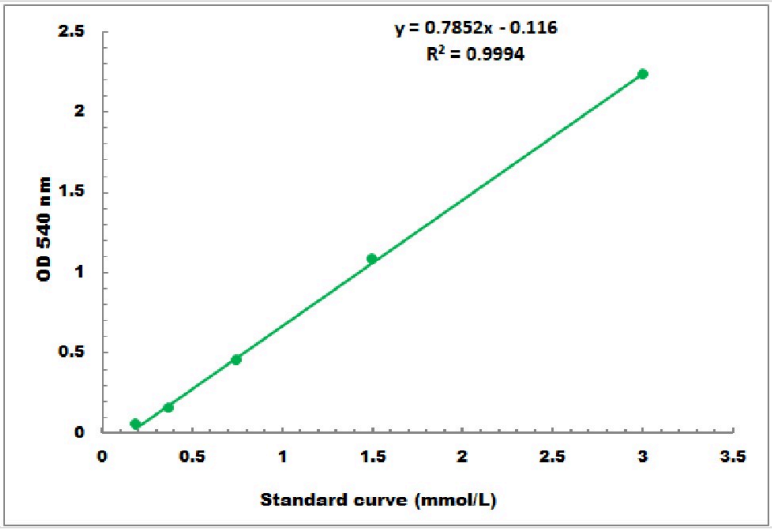
2.3According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V \times V_{Sample} / (V + V_{Assay})} \ (\mu mol/ml)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, mmol/L = μmol/ml
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl
V_{Assay}	the volume of Assay Buffer, μl
$C_{Protein}$	the sample protein concentration, mg/ml
W	the weight of sample, g
V	the volume of sample in sample preparation, ml

8. Typical Data

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range : 0.2mmol/L -3mmol/L