Pectin

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1222

(Version 1.4A)

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

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

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I. INTRODUCTION

Pectin, any of a group of water-soluble carbohydrate substances that are found in the cell wallsand intercellular tissues of certain 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 stage 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 preventing certain types of cancer.

PectinColorimetric Microplate Assay Kit provides a very sensitive and convenient means to measure pectincontent 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 pectincontent in the sample.

II.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	

III. 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

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 10 mmol/L of standardstock solution, store at 4 °C for 3-4 monthsor -20°C for 1 year after reconstitution.Then dilute to 3 mmol/Lstandard top solutionby adding0.3 ml stock solution into 0.7 mldistilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be3/1.5/0.75/0.375/0.188mmol/L.

Enzyme: Briefly centrifuge prior to opening. Add1 mlReaction 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.

V. 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 10minutes, 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 µlReaction Buffer into the microcentrifuge tube, mixfor detection.

2. For liquid samples

Add 10 µl sample and 190 µlReaction Buffer into the microcentrifuge tube, mix for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe microcentrifuge tubefirst:

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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe 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

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

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

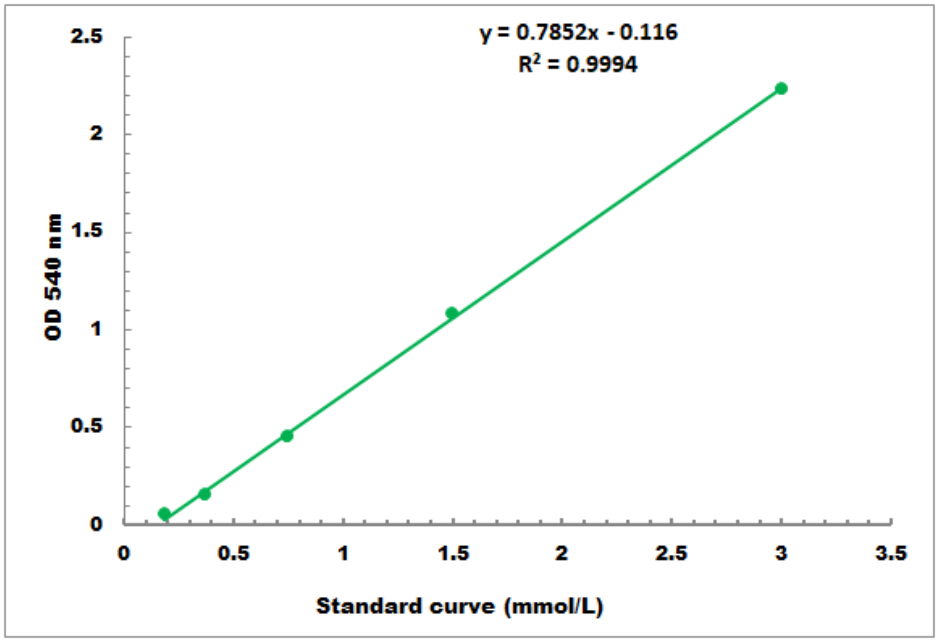
$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

V: the volume of sample in sample preparation, ml

VIII. 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