PectinMicroplate Assay Kit

User Manual

Catalog # CAK1222

(Version 1.3A)

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

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

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.

Pectin 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 react 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	
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Note:

Enzyme:add1 mlReaction Bufferto dissolve before use.

Standard: add 1 ml Distilled waterto dissolve before use; then add 0.3 ml into 0.7 ml Distilled water, mix,the concentration will be 3 mmol/L.

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. Convection oven

IV. 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 200 µlReaction Buffer into the microcentrifuge tube, mix for detection.

V. ASSAY PROCEDURE

Add following reagents intothe microcentrifuge tubefirst:

Reagent	Sample	Standard	Blank
Reaction Buffer	80 µl	--	--
Enzyme	10 µl		
Sample	10 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	--
Distilled water	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, put it into the convection oven, 90 °Cfor 10 minutes, record absorbance measured at 540nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

$$Pectin(\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Total})$$

$$= 6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the volume of sample

$$Pectin\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times V / V_{Total})$$

$$= 6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V$$

V_{Sample}: the volume of sampleinthe reaction, 0.01 ml;

$V_{Standard}$: the volume of standard, 0.1 ml;

V_{Total}: the volume of all sample, reaction buffer, 0.2 ml;

$C_{Standard}$: the standard concentration, 3mmol/L = 3μmol/ml;

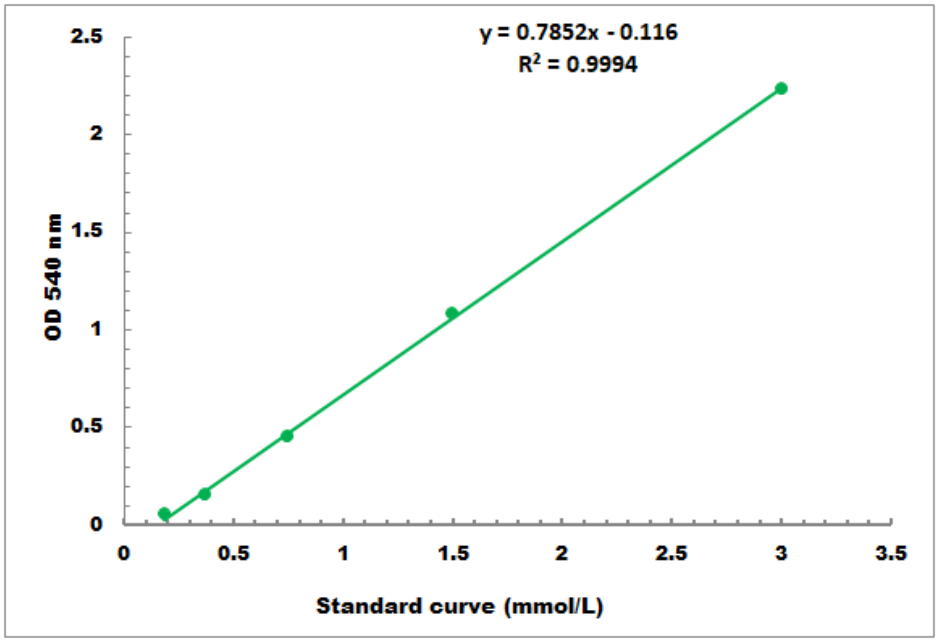
$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

V: the volume of sample, ml.

VII. TYPICAL DATA

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



Detection Range: 0.3mmol/L -3mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES