D-Galacturonic Acid Microplate Assay Kit User Manual

Catalog # FTA0200

(Version 1.2A)

Detection and Quantification of D-Galacturonic Acid Content in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

D-galacturonic acid is naturally occurring hexuronic acid present in glycosaminoglucans, glucuronide conjugates in mammals and in plant cell wall polysaccharides. D-galacturonic acid are major components of plant cell wall polysaccharides. D-galacturonic acid is the major component of pectin comprising the α-(1,4)-linked galacturonan backbone of homogalacturonan and rhamnogalacturonan II, and is present within the repeating disaccharide unit [,4)-α-D-GalpA-(1,2)-α- L-Rhap-(1,] of rhamnogalacturonan I.

D-Galacturonic Acid Microplate Assay Kit is designed to measure D-Galacturonic Acid directly in biological samples. The intensity of the color, measured at 525nm, is directly proportional to the D-Galacturonic Acid concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	15 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Dye Reagent: add 1 ml Dye Reagent Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.1 ml into 0.9 ml distilled water. The concentration will be 2.5 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 525 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 cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); incubate the solution at 90-95°C for 10 minutes; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer, put it in water bath of 80 °C for 30 minutes, centrifuged at 8,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Reaction Buffer	150 µl	150 µl	150 µl
Mix, cover the plate adhesive strips, put the plate into the convection oven, 90 °C for 20 minutes. When cold, add the Dye Reagent into the wells.			
Dye Reagent	10 µl	10 µl	10 µl
Mix, wait for 2 minutes, record absorbance measured at 525 nm.			

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.

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{D-galacturonic acid (μmol/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the quantity of cells or bacteria

$$\text{D-galacturonic acid (μmol/10}^4\text{ cell)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay})$$

$$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

3. According to the weight of sample

$$\text{D-galacturonic acid (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

4. According to the volume of sample

$$\text{D-galacturonic acid (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

V_{Sample}: the volume of sample, 0.02 ml;

$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

$C_{Standard}$: the standard concentration, 2.5 mmol/L = 2.5 μmol/ml;

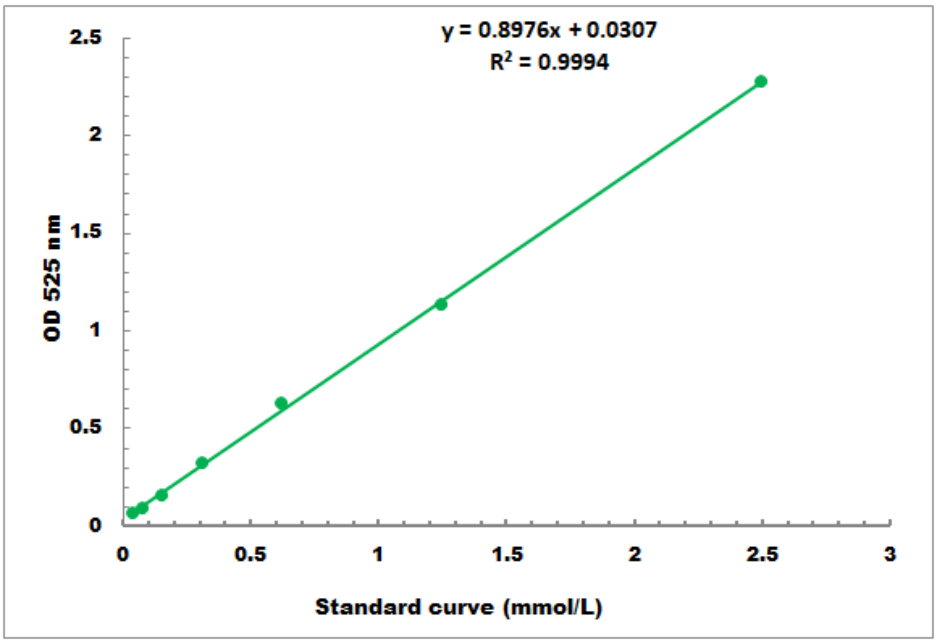
$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4.

VII. TYPICAL DATA

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



Detection Range: 0.05 mmol/L - 2.5 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES