



D-Galacturonic Acid Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1221

(Version 1.3A)

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

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

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I. INTRODUCTION

D-galacturonic acid is naturally occurring hexuronic acid present in glycosaminoglycans, 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 Colorimetric 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	Powderx 1	4 °C, keep in dark
Dye Reagent Diluent	1 ml x 1	4 °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 525 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. Dissolve in 1 ml distilled water to generate 25 mmol/L of standard stock solution, store at 4 °C for 1 month after reconstitution. Then dilute to 2.5 mmol/L standard top solution by adding 0.1 ml stock solution into 0.9 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 2.5/1.25/0.625/0.312/0.156/0.078/0.039 mmol/L.

Dye Reagent: Briefly centrifuge prior to opening. Add 1 ml Dye Reagent Diluent to dissolve before use. Keep in dark for immediate use or store at -20 °C for 1-2 weeks.

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

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 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.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

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

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

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mmol/L})$$

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 quantity of cells or bacteria

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol}/10^4)$$

2.2 According to the weight of sample

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

2.3 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, mmol/L = $\mu\text{mol/ml}$

V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

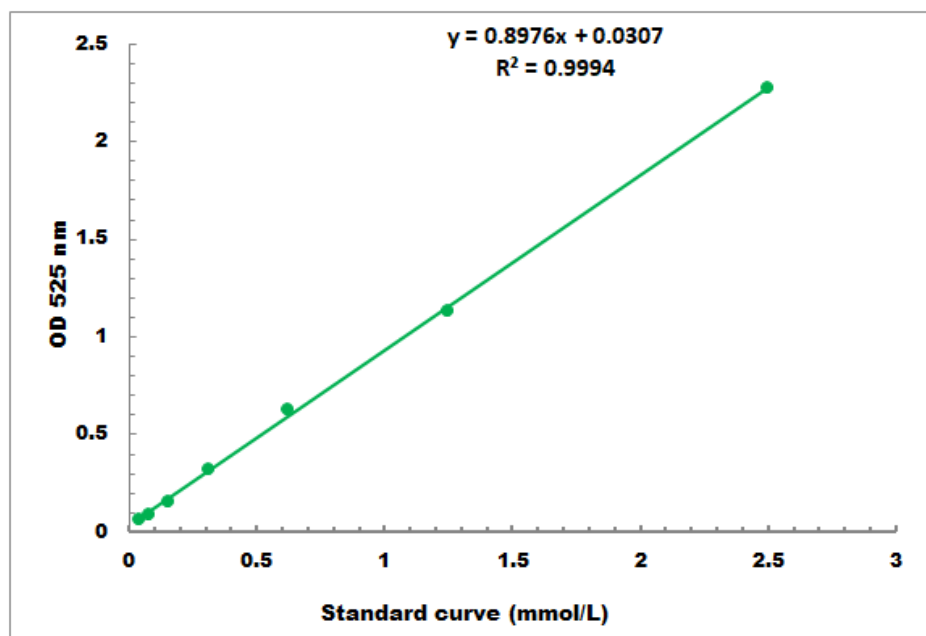
V_{Assay} : the volume of Assay Buffer, μl

W: the weight of sample, g

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

VIII. TYPICAL DATA

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



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