D-Galacturonic acid Assay Kit

ARG83414 D-Galacturonic acid Assay Kit can be used to measure D-Galacturonic acid in tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83414

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

d-Galacturonic acid is a sugar acid, an oxidized form of d-galactose. It is the main component of pectin, in which it exists as the polymer polygalacturonic acid. In its open form, it has an aldehyde group at C1 and a carboxylic acid group at C6. Other oxidized forms of d-galactose are d-galactonic acid (carboxylic group at C1) and meso-galactaric acid (mucic acid) (carboxylic groups at C1 and C6). It is also a uronic acid or hexuronic acid. Naturally occurring uronic acids are d-glucuronic acid, d-galacturonic acid, l-iduronic acid and d-mannuronic acid.

PRINCIPLE OF THE ASSAY

The D-Galacturonic acid Assay Kit can measure D-Galacturonic acid in tissue extracts, cell lysate, cell culture media and other biological fluids samples. The increase in absorbance at 525 nm is directly proportional to the D-Galacturonic Acid content.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Assay Buffer	4 X 30 ml	4°C
Reaction Buffer	15 ml (ready to use)	4°C
Reagent Dye	1 vial (lyophilized)	4°C
Reagent Dye Diluent	1 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 525 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- All component store at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and bacteria - Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 106 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.

Tissue - 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.

*Note: liquid samples can detect directly.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **distilled water** to dissolve standard; then add 0.1 ml into 0.9 ml distilled water. The concentration will be 2.5 mmol/L. Perform 2-fold serial dilution of the top standards to make the standard curve.

- **Reagent Dye**: add **1 ml Reagent Dye Diluent** to dissolve before assay.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **20 µl Sample** into Sample wells.
2. Standard wells: Add **20 µl Standard** into Standard wells.
3. Add **150 µl Reaction Buffer** to each wells.
4. Mix well. Incubate at **90°C** for **20 min**
5. Add **10 µl Reagent Dye** to each wells.
6. Mix well. Incubate at **RT** for **2 min**. Read the OD at **525nm**

Reagent	Sample	Standard	Blank
Sample	20 µl	-	-
Standard	-	20 µl	-
Distilled water	-		20 µl
Reagent Buffer	150 µl	150 µl	150 µl
Mix well. Incubate at **90°C** for **20 min**			
Reagent Dye	10 µl	10 µl	10 µl
Mix well. Incubate at **RT** for **2 min**. Read the OD at **525nm**			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

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

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

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 20 µl = 0.02 ml;

$V_{standard}$: the volume of standard, 20 µl = 0.2 ml;

V_{assay}: the volume of Assay Buffer, 1000 µl = 1 ml.

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

B. Formula:

a). According to the weight of sample

D-galacturonic acid (µmol/g) =

$[(C_{Standard} \text{ X } V_{standard}) \text{ X } (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \text{ X } (W \text{ X } V_{Sample} / V_{assay})]$

$=2.5 \text{ X } (OD_{Sample} - OD_{blank}) / [(OD_{Standard} - OD_{Blank}) \text{ X } W]$

b). According to the volume of sample

D-galacturonic acid (µmol/g) =

$[(C_{Standard} \text{ X } V_{standard}) \text{ X } (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \text{ X } V_{assay})]$

$=2.5 \text{ X } (OD_{Sample} - OD_{blank}) / (OD_{Standard} - OD_{Blank})$

c). According to the cell or bacteria

D-galacturonic acid (µmol /10^4 cell) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{Assay})]$

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

3. Detection range:

The detection range is from 0.05 mg/ml – 2.5 mg/ml.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.