D-Galacturonic Acid Assay Kit

Catalog No.: abx298938

Size: 96 Assays

Storage: Store all components at 4°C.

Application: For quantitative detection of D-Galacturonic Acid concentrations in tissue homogenates, cell lysates, cell culture supernatants and other biological fluids.

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

Introduction: D-galacturonic acid is naturally occurring hexuronic acid that is a major component of plant cell wall polysaccharides and pectin. Abbexa's D-Galacturonic Acid Assay Kit is a quick, convenient, and sensitive method for measuring and calculating D-Galacturonic Acid concentrations. The dye reagents react with D-Galacturonic Acid to create an absorption maximum at 525 nm. The intensity of the color is proportional to the concentration of D-Galacturonic Acid, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 15 ml
4. Dye Reagent: 1 vial
5. Dye Reagent Diluent: 1 ml
6. Standard: 1 vial
7. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (525 nm)
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Standard Solution**
  Add 1 ml of distilled water into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 100 µl of this solution and add 900 µl of distilled water to prepare the Standard Solution (concentration 2.5 mmol/L).

- **Dye Reagent Solution**
  Add 1 ml of Dye Reagent Diluent into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Incubate at 90-95°C for 10 minutes, then centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer, then incubate in a water bath at 80°C for 30 minutes. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

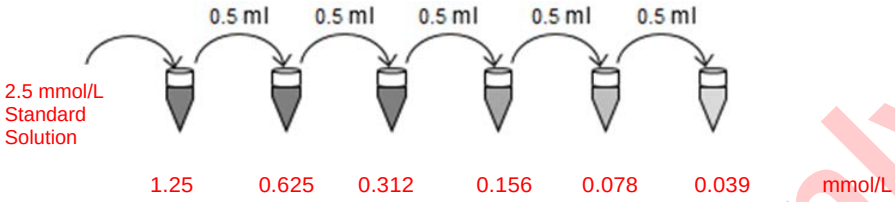
- **Liquid samples**
  Liquid samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 6 tubes with 1.25 mmol/L, 0.625 mmol/L, 0.3125 mmol/L, 0.156 mmol/L, 0.078 mmol/L, and 0.039 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 2.5 mmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 20 µl of sample to the sample wells.
4. Add 20 µl of prepared standards to the standard wells.
5. Add 20 µl of distilled water to the blank wells.
6. Add 150 µl of Reaction Buffer to all wells.
7. Tap the plate gently to mix. Cover the plate with a plate sealer, and incubate in a convection oven at 90°C for 20 minutes.
8. Allow the plate to cool, then add 10 µl of Dye Reagent Solution to all wells.
9. Tap the plate gently to mix. Allow to stand for 2 minutes. Read and record absorbance at 525 nm.

C. Calculations

D-galacturonic acid concentration per mg of protein:

$$\mathbf{D-galacturonic\ acid\ (\mu mol/mg)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times C_{Protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{2.5}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

D-galacturonic acid concentration per g of sample:

$$\mathbf{D-galacturonic\ acid\ (\mu mol/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{2.5}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

D-galacturonic acid concentration per 10^4 cells or bacteria:

$$\mathbf{D-galacturonic\ acid\ (\mu mol/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{2.5}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

D-galacturonic acid concentration per ml of sample:

$$\mathbf{D-galacturonic\ acid\ (\mu mol/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{2.5} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (2.5 mmol/L= 2.5 µmol/ml)
$\mathbf{W}$	Weight of the sample (g)
$\mathbf{N}$	Number of cells or bacteria ($\times 10^4$)
$\mathbf{V_{Assay}}$	Volume of Assay Buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.02 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.02 ml)

D. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.