Galactose (Gal) Assay Kit

Catalog No.: abx298927

Size: 100 Assays

Storage: Store all components in the dark at 4°C.

Application: For quantitative detection of Galactose (Gal) concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, saliva, milk and other biological fluids.

Detection Range: 0.01 mmol/L – 1 mmol/L

Introduction: Galactose ($C_6H_{12}O_6$) is a monosaccharide that is found in dairy products, sugar beets, gums and mucilages. It is also synthesized in mammals, where it forms part of glycolipids and glycoproteins in several tissues. It forms the disaccharide lactose when combined with glucose.

Abbexa's Galactose (Gal) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Gal concentrations. Galactose dehydrogenase catalyzes galactose and NAD to galactonic acid and NADH, inducing a colour change which can be measured spectrophotometrically at 450 nm. The intensity of the color is proportional to the concentration of Gal, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer I: 1 × 30 ml
3. Assay Buffer II: 1 x 30 ml
4. Reaction Buffer: 1 x 10 ml
5. Coenzyme: 1 vial
6. Enzyme: 1 x 50 µl
7. Dye Reagent A: 1 vial
8. Dye Reagent B: 1 x 1 ml
9. Standard: 1 vial
10. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Sonicator
6. Mortar
7. Water bath

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent Solution**
  Add 9 ml of Distilled Water into the Dye Reagent A vial and mix thoroughly to prepare the Dye Reagent Solution.
- **Coenzyme Solution**
  Add 1 ml Reaction Buffer and mix thoroughly to prepare the Coenzyme Solution. Ensure that the Coenzyme has completely dissolved prior to use.
- **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 1 mmol/L). Unused Standard Solution can be stored at 4°C.
- **Enzyme Solution**
  Add 1 ml Reaction Buffer and mix thoroughly to prepare the Enzyme Solution. Ensure that the Enzyme 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 0.5 ml of Distilled Water 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. Add 250 µl of Assay Buffer I and 250 µl of Assay Buffer II. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 0.5 ml of Assay Buffer on ice. Centrifuge at 12,000 × g at 4°C for 10 minutes. Add 250 µl of Assay Buffer I and 250 µl of Assay Buffer II Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

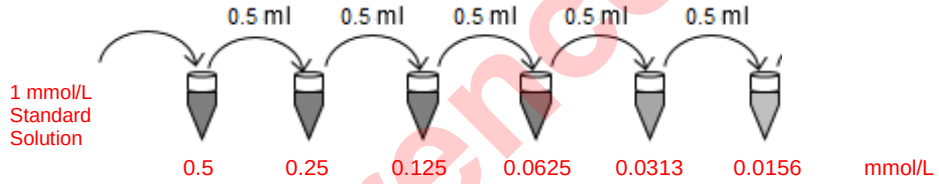
- **Liquid samples**
  Serum and plasma can be assayed directly. Liquid samples can be used directly. Milk samples should be cleared by mixing 500 µl sample with 250 µl Assay Buffer I and 250 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm. Transfer the supernatant into a clean tube for detection.

B. Assay Procedure

Warm all reagents to 37°C 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 7 tubes with 0.5 mmol/L, 0.25 mmol/L, 0.125 mmol/L, 0.0625 mmol/L, 0.0313 mmol/L and 0.0156 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 200 µmol/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 60 µl of Reaction Buffer to all wells.
4. Add 20 µl of sample to the sample wells.
5. Add 20 µl of prepared standards to the standard wells.
6. Add 20 µl of distilled water to the blank wells.
7. Add 10 µl of Coenzyme Solution to all wells.
8. Add 10 µl of Enzyme Solution to all wells.
9. Tap the plate gently to mix. Allow to stand for 30 minutes.
10. Add 90 µl of Dye Reagent A Solution to all wells
11. Add 10 µl of Dye Reagent B Solution to all wells.
12. Tap the plate gently to mix. Read and record absorbance at 450 nm.

C. Calculations

Galactose concentration per mg of protein:

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

Galactose concentration per 10^4 cells or bacteria:

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

Galactose concentration per ml:

$$\mathbf{Gal\ (\mu mol/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{2} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$C_{Standard}$	Concentration of highest standard (1 mmol/L)
W	Weight of the sample (in g)
N	Number of cells or bacteria ($\times 10^4$)
V_{Assay}	Volume of Distilled Water, Assay Buffer I and Assay Buffer II (1 ml)
V_{Sample}	Volume of sample (0.02 ml)
$V_{Standard}$	Volume of standard (0.02 ml)