EnzyChrom™ Galactose Assay Kit (EGAL-100)

Quantitative Colorimetric Galactose Determination

DESCRIPTION

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. Simple, direct and high-throughput assays for galactose determination find wide applications. BioAssay Systems' assay uses specific enzyme-coupled reactions to form a colored product. The color intensity at 570nm or fluorescence intensity at 530nm/585nm is directly proportional to the galactose concentration in the sample.

KEY FEATURES

Use as little as 20 µL samples. Linear detection range in 96-well plate: 10 to 1000 µM galactose for colorimetric assays and 10 to 100 µM for fluorimetric assays.

APPLICATIONS:

Direct Assays: galactose in serum, plasma, urine, saliva, milk, culture medium and other biological samples.
Drug Discovery/Pharmacology: effects of drugs on galactose metabolism.
Food and Beverages: galactose in food and beverages products.

KIT CONTENTS:

Assay Buffer: 10 mL **Enzyme Mix:** 120 µL
Dye Reagent: 120 µL **Standard**: 1 mL 10 mM Galactose

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of three months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIEMTRIC PROCEDURE

Note: (1) glycerol and SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation. (2) This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample treatment: serum and plasma samples can be assayed directly. Milk samples should be cleared by mixing 600 µL milk with 100 µL 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor *n* = 1.36).

1. Equilibrate all components to room temperature. During experiment, keep thawed Enzyme Mix in a refrigerator or on ice.

2. Standards and samples: prepare 400 µL 1000 µM Standard by mixing 40 µL 10 mM standard with 360 µL dH_2O. Dilute standard in dH_2O as follows.

No	1000 µM STD + H_2O	Vol (µL)	Galactose (µM)
1	100 µL + 0 µL	100	1000
2	80 µL + 20 µL	100	800
3	60 µL + 40 µL	100	600
4	40 µL + 60 µL	100	400
5	30 µL + 70 µL	100	300
6	20 µL + 80 µL	100	200
7	10 µL + 90 µL	100	100
8	0 µL +100 µL	100	0

Transfer 20 µL standards and 20 µL samples into separate wells of a clear flat-bottom 96-well plate.

3. *Reaction*. For each reaction well, mix 85 µL Assay Buffer, 1 µL Enzyme Mix (*vortex briefly before pipetting*), and 1 µL Dye Reagent in a clean tube. Transfer 80 µL Working Reagent into each reaction well. Tap plate to mix. Incubate 20 min at room temperature.

4. Read optical density at 570nm (550-585nm).

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 10 to 100 µM galactose. Prepare 100 µM galactose standard by mixing 10 µL 10 mM standard with 990 µL H_2O. Then dilute standards in H_2O (see *Colorimetric Procedure*) to 100, 80, 60, 40, 30, 20, 10 and 0 µM.

1. Transfer 20 µL standards and 20 µL samples into separate wells of a *black* 96-well plate.

2. Add 80 µL Working Reagent, tap plate to mix. Incubate 20 min.

3. Read fluorescence at λ_{ex} = 530nm and λ_{em} = 585nm.

Notes: If the calculated galactose concentration of a sample is higher than 1000 µM in colorimetric assay or 100 µM in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor n.

CALCULATION

Subtract blank value (water, #8) from the standard values and plot the ΔOD or ΔRFU against standard concentrations. Determine the slope and calculate the galactose concentration of Sample,

Colorimetry: $$[\text{Galactose}] = \frac{OD_{SAMPLE} - OD_{H2O}}{\text{Slope}} \times n \quad (\mu M)$$

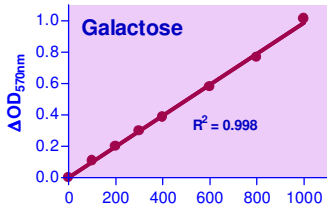
Fluorimetry: $$[\text{Galactose}] = \frac{RFU_{SAMPLE} - RFU_{H2O}}{\text{Slope}} \times n \quad (\mu M)$$

OD_{SAMPLE}, OD_{H2O} are optical density values of the sample and water. RFU_{SAMPLE}, RFU_{H2O} are fluorescence intensity values of the sample and water. n is the dilution factor.
Conversions: 1 mM galactose equals 18 mg/dL, 0.018% or 180 ppm.

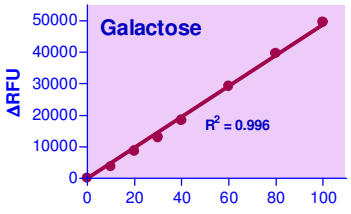
MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates, optical density plate reader; black 96-well plates and fluorescence plate reader.

Galactose Standard Curves



96-well colorimetric assay



96-well fluorimetric assay

LITERATURE

1. Novelli G, Reichardt JK. (2000). Molecular basis of disorders of human galactose metabolism: past, present, and future. Mol Genet Metab. 71:62-65.

2. Pudek MR et al. (1990). Low concentration galactose determination in plasma adapted to the Cobas-Bio. Clin Biochem. 23:221-223.

3. Gabrielli M. (1978). Serum galactose determination with centrifugal analyzers. Clin. Chem. 24:1990-1995.