Galactose Assay Kit (Colorimetric)

Galactose Assay Kit (Colorimetric) is a detection kit for the quantification of Galactose in serum, plasma, urine, saliva, milk, culture medium, food and beverage.

Catalog number: ARG82160

Package: 100 tests

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INTRODUCTION

Galactose (milk sugar) sometimes abbreviated Gal, is a monosaccharide sugar that is about as sweet as glucose, and about 65% as sweet as sucrose. It is an aldohexose and a C-4 epimer of glucose. A galactose molecule linked with a glucose molecule forms a lactose molecule.

Galactan is a polymeric form of galactose found in hemicellulose, and forming the core of the galactans, a class of natural polymeric carbohydrates. [Provide by Wikipedia: Galactose]

PRINCIPLE OF THE ASSAY

This Galactose Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of galactose present in biological samples. This assay uses specific enzyme-coupled reactions to form a colored product. The color intensity at O.D. 570 nm or fluorescence intensity at 530 nm/585 nm is directly proportional to the galactose concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on dry ice. Store all components at -20°C upon receiving. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Enzyme Mix (lyophilized)	1 vial	-20°C
Dye Reagent	120 µL	-20°C
Standard (10 mM D-galactose)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- 6N HCl / NaOH
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Glycerol and SH-containing reagents (E.g., β–mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation.
- 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.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in 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.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

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

Other liquid biological sample: Assay directly.

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.

REAGENT PREPARATION

- **Reconstitute Enzyme Mix:** adding 120 µL of distilled water to the Enzyme tube. Make sure Enzyme Mix is fully dissolved by pipetting up and down. Store reconstituted Enzyme at-20°C and use within 3 months.
- **Working Reagent:** for each assay, mix 85 µL of Assay Buffer, 1 µL of Reconstituted Enzyme Mix, 1 µL of Dye Reagent in a clean tube. Prepare immediately before assay.
- **Standards:** mix 40 µL of 10 mM Standard with 360 µL of distilled water (final 1000 µM). Dilute standards as follows.

Standard tube	Galactose (µM)	Distilled water (µL)	Standard Premix, 1000 µM (µL)
S1	1000	0	100
S2	800	20	80
S3	600	40	60
S4	400	60	40
S5	300	70	30
S6	200	80	20
S7	100	90	10
S0	0	100	0

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep reconstituted Enzyme Mix tube on ice during assay.

COLORIEMTRIC PROCEDURE

	Standard well	Sample well
Standard	20 µL	
Sample		20 µL
Working Reagent	80 µL	80 µL
Tap plate to mix briefly and thoroughly. Incubate for **20 minutes** at **room temperature**.		
Read the absorbance at **O.D. 570 nm**. (550-585 nm)		

FLUORIMETRIC PROCEDURE

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

Note: 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 distilled water and repeat the assay. Multiply result by the dilution factor n.

CALCULATION OF RESULTS

1. Subtract blank value (distilled water, S8) from the standard values and plot the ΔOD or ΔRFU against standard concentrations. Determine the slope and calculate the galactose concentration of Sample as follows:

   Galactose (µM) = [(R_{Sample} – R_{Blank}) / Slope (μM^{-1})] x n

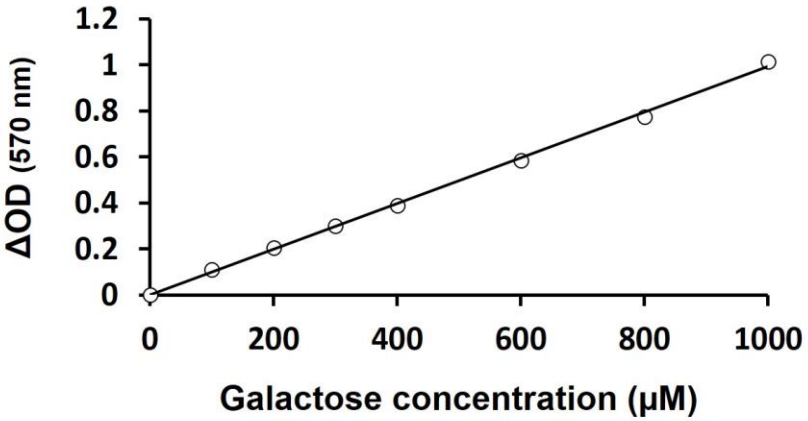
   Note:

   - R_{Sample}, R_{Blank}: the O.D. 565 nm values and fluorescence intensity values of the sample and distilled water blank.
   - n is the sample dilution factor.

2. Conversions: 1 mM galactose equals 18 mg/dL, 0.018% or 180 ppm.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Galactose Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

10 μM