

Galactose Assay Kit (Colorimetric/Fluorometric)

LS-K171-100 (100 Tests) • Store at -20°C



Introduction

GALACTOSE (C₆H₁₂O₆) 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. LSBio's 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 fluorometric 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.

Components

Component	K171-100
	100 Tests
Assay Buffer	10 mL
Enzyme Mix	Dried
Dye Reagent	120 µL
Standard	1 mL

Materials Not Supplied

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

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

FOR RESEARCH USE ONLY! Not for use in humans.

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Assay Procedure

Colorimetric 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 pipette is recommended.

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

1. Equilibrate all components to room temperature. Reconstitute the Enzyme mix with 120 μ L dH₂O. Reconstituted Enzyme mix is stable for 3 months if stored at -20°C. During experiment, keep reconstituted Enzyme Mix in a refrigerator or on ice.
2. Standards and samples: prepare 400 μ L 1000 μ M Standard by mixing 40 μ L 10 mM standard with 360 μ L dH₂O. Dilute standard in dH₂O as follows.

No	1000 μ M STD + H ₂ O	Vol (μ L)	Galactose (μ M)
1	100 μ L + 0 μ L	100	1000
2	80 μ L + 20 μ L	100	800
3	60 μ L + 40 μ L	100	600
4	40 μ L + 60 μ L	100	400
5	30 μ L + 70 μ L	100	300
6	20 μ L + 80 μ L	100	200
7	10 μ L + 90 μ L	100	100
8	0 μ L + 100 μ L	100	0

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

3. Reaction. For each reaction well, mix 85 μ L Assay Buffer, 1 μ L Enzyme Mix (vortex briefly before pipetting), and 1 μ L Dye Reagent in a clean tube. Transfer 80 μ L Working Reagent into each reaction well. Tap plate to mix. Incubate 20 min at room temperature.
4. Read optical density at 570nm (550-585nm).

Fluorometric Procedure

For fluorometric assays, the linear detection range is 10 to 100 μ M galactose. Prepare 100 μ M galactose standard by mixing 10 μ L 10 mM standard with 990 μ L H₂O. Then dilute standards in H₂O (see Colorimetric Procedure) to 100, 80, 60, 40, 30, 20, 10 and 0 μ M.

1. Transfer 20 μ L standards and 20 μ L samples into separate wells of a black 96-well plate.
2. Add 80 μ L Working Reagent, tap plate to mix. Incubate 20 min.
3. Read fluorescence at $\lambda_{ex} = 530$ nm and $\lambda_{em} = 585$ nm.

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

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Calculations

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,

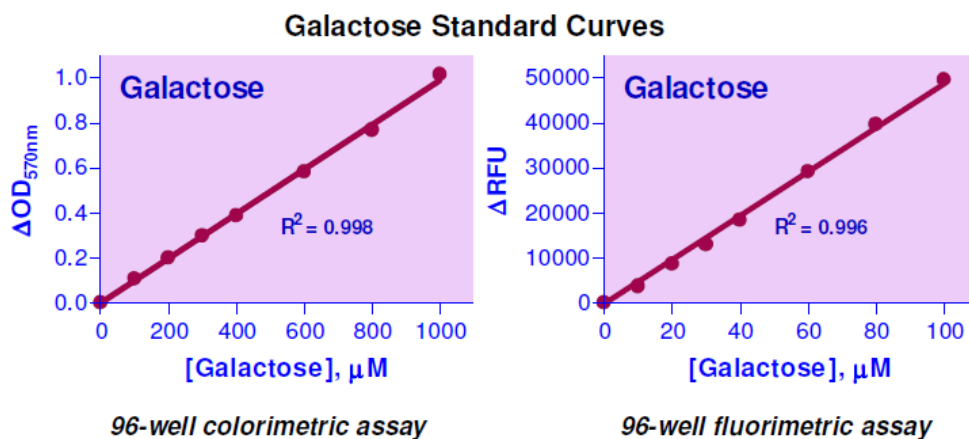
$$\text{Colorimetry: } [\text{Galactose}] = \frac{OD_{\text{SAMPLE}} - OD_{\text{H}_2\text{O}}}{\text{Slope}} \times n \quad (\mu\text{M})$$

$$\text{Fluorimetry: } [\text{Galactose}] = \frac{RFU_{\text{SAMPLE}} - RFU_{\text{H}_2\text{O}}}{\text{Slope}} \times n \quad (\mu\text{M})$$

OD_{SAMPLE} , $OD_{\text{H}_2\text{O}}$ are optical density values of the sample and water. RFU_{SAMPLE} , $RFU_{\text{H}_2\text{O}}$ 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.

Sample Data



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