Technical Bulletin

Galactose Assay Kit

Catalogue number MAK502

Product Description

Galactose ($C_6H_{12}O_6$) is a monosaccharide that is found in dairy products, sugar beets, gums and mucilage's. 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. The Galactose Assay Kit uses specific enzyme-coupled reactions to form a colored product. The absorbance at 570 nm or fluorescence intensity at λ_{Ex} =530 nm/λ_{Em} =585 nm is directly proportional to the galactose concentration in the sample.

The linear detection range of the kit is 10 to 1000 µM galactose for colorimetric assays and 10 to 100 µM for fluorometric assays. The kit is suitable for galactose activity determination serum, plasma, urine, saliva, milk, culture medium, food, beverages products and other biological samples, as well as for studying the effects of drugs on galactose metabolism.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer — 10 mL
  Catalogue Number MAK502A
- Enzyme Mix — 1 vial
  Catalogue Number MAK502B
- Dye reagent — 120 µL
  Catalogue Number MAK502C
- Standard (10 mM) — 1 mL
  Catalogue Number MAK502D

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor).
- Multiwell plate reader.
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Enzyme mix

Reconstitute the Enzyme mix with 120 µL 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.

Equilibrate all other components to room temperature prior to use.

Briefly vortex the enzyme mx before pipetting.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Note: Glycerol and SH-containing reagents (for example: 2–mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in Sample preparation.

Serum and plasma Samples can be assayed directly.

Milk Samples should be cleared by:

1. Mixing 600 µL milk with 100 µL 6 N HCl.
2. Centrifuge 5 minutes at 14,000 rpm.
3. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 N NaOH.
4. The neutralized supernatant is ready for assay (dilution factor (DF) = 1.36).

Colorimetric Standard Curve Preparation

1. Prepare a 1000 µM Standard by mixing 40 µL of the 10 mM Standard with 360 µL purified water.
2. Prepare Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.

Preparation of Galactose Colorimetric Standards

No.	1000 µM Standard	Purified Water	Galactose (µM)
1	100 µL	0 µL	1000
2	80 µL	20 µL	800
3	60 µL	40 µL	600
4	40 µL	60 µL	400
5	30 µL	70 µL	300
6	20 µL	80 µL	200
7	10 µL	90 µL	100
8	0 µL	100 µL	0

3. Transfer 20 µL Standards and 20 µL Samples into separate wells of a clear flat-bottom 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare 100 µM galactose Standard by mixing 10 µL 10 mM Standard with 990 µL purified water.
2. Prepare Standards in 1.5 mL microcentrifuge tubes according to Table 2.

Table 2.

Preparation of Galactose Fluorometric Standards

No.	100 µM Standard	Purified Water	Galactose (µM)
1	100 µL	0 µL	100
2	80 µL	20 µL	80
3	60 µL	40 µL	60
4	40 µL	60 µL	40
5	30 µL	70 µL	30
6	20 µL	80 µL	20
7	10 µL	90 µL	10
8	0 µL	100 µL	0

3. Transfer 20 µL Standards and 20 µL Samples into separate wells of a black flat-bottom 96-well plate.

Working Reagent Preparation

Mix enough reagents for the number of assays to be performed. For each Standard and Sample well, prepare 87 µL of Working Reagent according to Table 3.

Table 3.

Preparation of Working Reagents

Reagent	Working Reagent
Assay Buffer	85 µL
Enzyme mix	1 µL
Dye reagent	1 µL

Note: 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.

Measurement

1. Incubate the plate for 20 minutes at room temperature.
2. Measure optical density at 570 nm (550-585 nm) or fluorescence intensity at λ_{Ex} = 530 nm/ λ_{Em} = 585 nm.

Results

1. Subtract the water blank (Std #8) value from all the Standard values.
2. Plot the ΔRFU or ΔOD against the Standard concentrations.
3. Determine the slope and calculate galactose concentration of samples using the below given equation:

$$\text{Galactose } (\mu M) = \frac{R_{Sample} - R_{Blank}}{\text{Slope } (\mu M^{-1})} \times \times DF$$

Where:
R_{SAMPLE} = OD or fluorescence intensity (F) reading of Sample
R_{BLANK} = OD or fluorescence intensity (F) reading of Sample Blank
DF = Sample dilution factor (DF = 1 for undiluted Samples)

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

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

Figure 1.

Typical Colorimetric Galactose Standard Curve

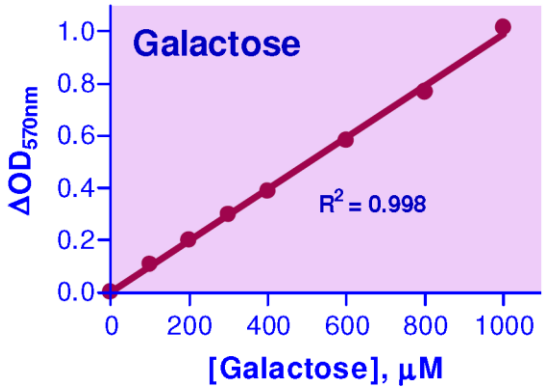
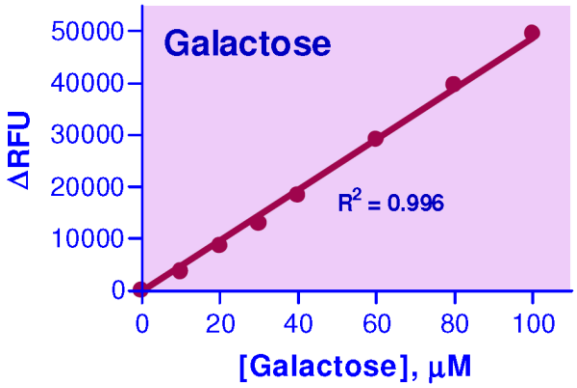


Figure 2.

Typical Fluorometric Galactose Standard Curve



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