USER GUIDE

For Research Use Only. Not For Use In Diagnostic Procedures

Version 3.0

半乳糖检测试剂盒

Galactose Assay Kit

Do not eat Store at -20° C & in the dark

Cat.No. MGK0784 **Size :** 100 tests

Technical literature is available at: www.mesgenbio.com.

E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com

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. MesGen Biotechnology 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 10 µL samples. Linear detection range in 96-well plate: 10 to 2500 µM galactose for colorimetric assays and 2 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

Reagent A : 5 mL

Reagent B : 5 mL

Reagent C : 200 µL

10mM **Galactose Standard:** 2 mL

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 reconstituted Reagent in a refrigerator or on ice.
2. *Standards and samples*: prepare 400 µL 2000 µM Standard by mixing 200 µL 10 mM standard with 800 µL dH_2O. Dilute standard in dH_2O as follows.

No.	2000 µM STD + H_2O	Vol (µL)	Galactose (µM)
1	100 + 0	100	2000
2	80 + 20	100	1600
3	60 + 40	100	1200
4	40 +60	100	800
5	10 + 90	100	200
6	5 + 95	100	100
7	2 + 98	100	40
8	1 + 99	100	20
9	0 + 100	100	0

Transfer 10 µL standards and 10 µL samples into separate wells of a clear flat-bottom 96-well plate.

3. *Reaction*. For each reaction well, mix 50 µL Reagent A, 50 µL Reagent B and and 2 µL Reagent C (*vortex briefly before pipetting*). Transfer 100 µL Working Reagent into each reaction well. Tap plate to mix.Incubate 5-10 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 10 µL standards and 10 µL samples into separate wells of a *black* 96-well plate.
2. Add 100 µL Working Reagent, tap plate to mix. Incubate 10-15 min.
3. Read fluorescence at λex = 530nm and λem = 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 from the standard values and plot the ΔOD or ΔRFU against standard concentrations. Determine the slope and calculate the galactose concentration of Sample,

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

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

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.

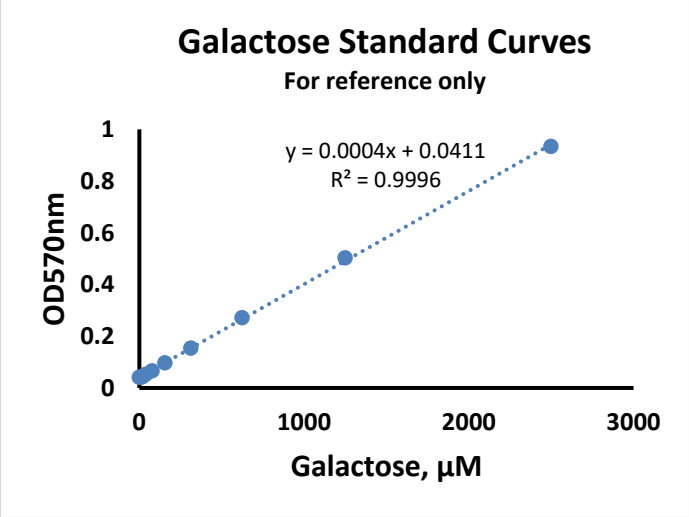
Conversions: 1 mM galactose equals 18 mg/dL, 0.018% or 180 ppm.

Storage conditions

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

Shelf life

3 months after receipt.



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