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Product Information

Galactose Colorimetric/Fluorometric Assay Kit

Catalog Number **MAK012**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Galactose is a simple monosaccharide that serves as an energy source and as an essential component of glycolipids and glycoproteins. Galactose contributes to energy metabolism via its conversion to glucose by the enzymes that constitute the Leloir pathway. Defects in the genes encoding these proteins lead to the metabolic disorder galactosemia.

In this assay kit, Galactose is oxidized resulting in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535 nm/ λ_{em} = 587 nm) product, proportional to the galactose present. This kit has a linear range of detection between 0.2–1.0 nmole galactose for the fluorometric assay and 2–10 nmole galactose for the colorimetric assay.

This kit is suitable for use with various biological samples including serum, plasma, other body fluids, food, and growth media.

Components

The kit is sufficient for 100 assays in 96 well plates.

Galactose Assay Buffer Catalog Number MAK012A	25 mL
Galactose Probe, in DMSO Catalog Number MAK012B	0.2 mL
Galactose Enzyme Mix Catalog Number MAK012C	1 vl
Horseradish Peroxidase (HRP) Catalog Number MAK012D	1 vl
Galactose Standard, 100 nmole/µL Catalog Number MAK012E	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader.

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.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Galactose Assay Buffer – Allow buffer to come to room temperature before use.

Galactose Probe – Ready-to-use as supplied. Allow Galactose Probe to come to room temperature before use. Store protected from light at –20 °C for use within 2 months.

Galactose Enzyme Mix and Horseradish Peroxidase – Reconstitute each in 220 µL of Galactose Assay Buffer. Mix well by pipetting, then aliquot each and store protected from light at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Galactose Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM (100 nmole/µL) Galactose Standard Solution with 990 µL of Galactose Assay Buffer to prepare a 1 mM (1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM Galactose standard solution into a 96 well plate, generating 0 (assay blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Galactose Assay Buffer to each well to bring the volume to 50 µL.

Galactose Standards for Fluorometric Detection
Prepare a 1 mM standard solution as for the Colorimetric Assay. Dilute 20 µL of the 1 mM standard solution with 180 µL of Galactose Assay Buffer to generate a 0.1 mM (0.1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM Galactose standard solution into a 96 well plate generating, 0 (assay blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Galactose Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Liquid samples can be measured directly. Bring samples to a final volume of 50 µL with Galactose Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Set up the Master Reaction Mixes according to the scheme in Tables 1 or 2. 50 µL of the appropriate Master Reaction Mix is required for each reaction (well). The fluorometric assay is ~10 fold more sensitive than the colorimetric assay and requires less probe reagent.

Table 1.
Colorimetric Master Reaction Mix

Reagent	Volume
Galactose Assay Buffer	44 µL
Galactose Probe	2 µL
Galactose Enzyme Mix	2 µL
HRP	2 µL

Table 2.
Fluorometric Master Reaction Mix

Reagent	Volume
Galactose Assay Buffer	45.6 µL
Galactose Probe	0.4 µL
Galactose Enzyme Mix	2 µL
HRP	2 µL

2. Add 50 µL of the appropriate Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 590 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (assay blank) Galactose Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Galactose standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Galactose

$$S_a/S_v = C$$

S_a = Amount of Galactose in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of Galactose in sample

Galactose molecular weight: 180.16 g/mole

Sample Calculation

Amount of Galactose (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50.0 μL

Concentration of Galactose in sample

$$5.84 \text{ nmole}/50.0 \ \mu\text{L} = 0.117 \text{ nmole}/\mu\text{L}$$

$$0.114 \text{ nmole}/\mu\text{L} \times 180.16 \text{ ng/nmole} = 21.1 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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