

## Product Information

### Galactose and Lactose Colorimetric/Fluorometric Assay Kit

Catalog Number **MAK011**  
Storage Temperature  $-20^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

Lactose is a disaccharide containing one galactose and one glucose molecule. Lactose is the major sugar in the milk of most species, typically present between 2–8%. The enzyme lactase hydrolyzes lactose to its constituent monosaccharides. 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 leads to the metabolic disorder galactosemia.

In this assay kit, galactose is oxidized by galactose oxidase resulting in a colorimetric (570 nm)/fluorometric ( $\lambda_{\text{ex}} = 535 \text{ nm}/\lambda_{\text{em}} = 587 \text{ nm}$ ) product, proportional to the galactose present. To detect lactose, lactose is hydrolyzed by lactase to generate free galactose, which is then measured (total galactose). The lactose level is equal to total galactose minus free galactose.

This kit is suitable for use with various biological samples including serum, plasma, other body fluids, food, and growth media. This kit has a linear detection range of 0.2–1.0 nmole galactose for the fluorometric assay and 2–10 nmoles galactose for the colorimetric assay.

### Components

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

Galactose Assay Buffer Catalog Number MAK011A	25 mL
Galactose Probe, in DMSO Catalog Number MAK011B	0.2 mL
Lactase Catalog Number MAK011C	1 vL

Galactose Enzyme Mix Catalog Number MAK011D	1 vL
Horseradish Peroxidase Catalog Number MAK011E	1 vL
Galactose Standard, 100 nmole/ $\mu\text{L}$ Catalog Number MAK011F	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

Allow reagents to come to room temperature and 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 probe to come to room temperature before use. Store protected from light and moisture at  $-20^{\circ}\text{C}$ . Use within 2 months. Upon thawing, the Galactose Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Galactose Probe Solution 5 to 10-fold with Galactose Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Lactase, Galactase Enzyme Mix, Horseradish Peroxidase – Reconstitute each in 220  $\mu\text{L}$  Galactose Assay Buffer. Mix well by pipetting, then aliquot each and store, protected from light, at  $-20\text{ }^{\circ}\text{C}$ . Use within 2 months of reconstitution.

### Storage/Stability

The kit is shipped on wet ice. Storage at  $-20\text{ }^{\circ}\text{C}$ , protected from light, is recommended.

### Procedure

All samples and standards should be run in duplicate.

#### Galactose Standards for Colorimetric Detection

**Note:** Prepare the diluted standard fresh and do not store.

Immediately prior to use, dilute 10 mL of the 100 mM (100 nmole/mL) Galactose Standard Solution with 990 mL of Galactose Assay Buffer to prepare a 1 mM (1 nmole/mL) standard solution. Mix well and add 0, 2, 4, 6, 8, and 10  $\mu\text{L}$  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  $\mu\text{L}$ .

#### Galactose Standards for Fluorometric Detection

Prepare a 1 mM Galactose standard solutions as for the colorimetric assay. Dilute 20  $\mu\text{L}$  of the 1 mM standard solution with 180  $\mu\text{L}$  of Galactose Assay Buffer to generate a 0.1 mM (0.1 nmole/ $\mu\text{L}$ ) standard solution. Add 0, 2, 4, 6, 8, and 10  $\mu\text{L}$  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  $\mu\text{L}$ .

#### Sample Preparation

Liquid samples can be directly added to the wells. Milk typically contains between 2–8% lactose. Add 0.01–0.1  $\mu\text{L}$  of milk samples to the wells. Bring all samples to a final volume of 50  $\mu\text{L}$  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

**Note:** To detect lactose, prepare two wells for each sample. Add 2  $\mu\text{L}$  of Lactase to one well to convert lactose to galactose and glucose (total galactose detection). Do not add lactase to the other well (free galactose detection). Incubate at  $37\text{ }^{\circ}\text{C}$  for 30 minutes.

1. Set up the Master Reaction Mix according to the scheme in Table 1. 50  $\mu\text{L}$  of the Master Reaction Mix is required for each reaction (well).

**Table 1.**

Master Reaction Mix

Reagent	Volume
Galactose Assay Buffer	44 $\mu\text{L}$
Galactose Probe	2 $\mu\text{L}$
Galactose Enzyme Mix	2 $\mu\text{L}$
HRP	2 $\mu\text{L}$

2. Add 50  $\mu\text{L}$  of the Master Reaction Mix to each well containing the Galactose Standard and test samples. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at  $37\text{ }^{\circ}\text{C}$ . 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 ( $\lambda_{\text{ex}} = 535/\lambda_{\text{em}} = 590\text{ nm}$ ).

## Results

### Calculations

The background for the assays 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 ( $\mu\text{L}$ ) added into the wells

$C$  = Concentration of Galactose in sample

### Sample Calculation

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

Sample volume ( $S_v$ ) = 50  $\mu\text{L}$

Molecular weight of galactose = 180.16

Concentration of Galactose in sample

$$5.84 \text{ nmole}/50 \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 180.16 \text{ ng/nmole} = 21.04 \text{ ng}/\mu\text{L}$$

Lactose = Total Galactose – Free Galactose

**Troubleshooting Guide**

<b>Problem</b>	<b>Possible Cause</b>	<b>Suggested Solution</b>
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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