3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Lactose Assay Kit

Catalog Number **MAK017**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Lactose is a dissacharide formed by the condensation of 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. In neonates, glucose released via the action of lactase is a major energy source.

In this assay, lactose concentration is determined by an enzymatic assay, in which lactose is converted to galactose and glucose. The galactose is subsequently oxidized, resulting in a colorimetric (570 nm)/ fluorometric (λ_{ex} = 535 nm/λ_{em} = 587 nm) product, proportional to the lactose initially present. This kit is suitable for use with various biological samples and has a linear range of detection between 0.2–1.0 nmole lactose for the fluorometric assay and 2–10 nmoles lactose for the colorimetric assay.

Components

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

Lactose Assay Buffer Catalog Number MAK017A	25 mL
Probe in DMSO Catalog Number MAK017B	0.2 mL
Lactase Catalog Number MAK017C	1 vl
Lactose Enzyme Mix Catalog Number MAK017D	1 vl
Horseradish Peroxidase (HRP) Catalog Number MAK017E	1 vl
Lactose Standard, 100 nMole/µL Catalog Number MAK017F	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

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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.

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

Probe – Warm to room temperature before use. Protect from light. Mix well by pipetting, then aliquot and store at –20 °C.

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

Lactase, Lactose Enzyme Mix, and HRP – Reconstitute each in 220 µL of Lactose Assay Buffer. Mix well by pipetting, then aliquot each and store at –20 °C. Use within 2 months of reconstitution. Keep cold while in use.

Storage/Stability

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

Procedure
All samples and standards should be run in duplicate.

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

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

Sample Preparation
Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well).

Tissue or cells can be homogenized in 4 volumes of the Lactose Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.

Serum samples (0.5–10 µL/assay) can be assayed directly by adding in duplicate to 96 well plate.

Bring samples to final volume of 50 µL/well with Lactose Assay Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Note: Free galactose present in the samples can generate background signal. To remove the effect of galactose background, a sample blank may be set up for each sample.

Reaction
1. Add 2 µL of Lactase to each of the standard and sample wells to convert the lactase to galactose. Add 2 µL of Lactose Assay Buffer to each of the sample blank wells.

2. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	**Samples, Standards, and Sample Blanks**
Lactose Assay Buffer	44 µL
Probe	2 µL
Lactose Enzyme Mix	2 µL
HRP	2 µL

3. Add 50 µL of the Master Reaction Mix to each of the wells (standards, samples, and sample blanks). Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at 37 °C. Protect the plate from light during the incubation.

4. For colorometric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 lactose standard (blank). Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate lactose standards to plot a standard curve. The amount of lactose present in the samples may be determined from the standard curve.

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

Subtract the sample blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of lactose present in the samples may be determined from the standard curve.

Concentration of Lactose

$$S_a/S_v = C$$

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

S_v = Sample volume (μL) added into the wells.

C = Concentration of lactose acid in sample

Lactose molecular weight is 342.3 g/mole.

Sample Calculation

Amount of Lactose (S_a) = 5.07 nmole

Sample volume (S_v) = 50 μL

Concentration of lactose in sample

5.07 nmole/50 μL = 0.101 nmole/μL

0.101 nmole/μL × 342.3 ng/nmole= 34.57 ng/μL

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	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 they 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

LS,MAM 06/13-1