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Product Information

High Sensitivity Lactulose Assay Kit

Catalog Number **MAK182**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Lactulose is a synthetic disaccharide derived from galactose and fructose. It is not absorbed in the gut and is frequently used in the treatment of constipation and hepatic encephalopathy. Studies have reported that lactulose administration may cause an increase in fecal nitrogen.[1] It is also known to function as a prebiotic.[2] Lactulose measurements can be used to detect gastrointestinal dysfunctions.

This Lactulose Assay Kit is a highly sensitive assay for determining lactulose levels (ranging from 200–1000 pmole/well) in a variety of samples such as food and dairy products. Lactulose concentration is determined by a coupled enzyme assay, which results in a fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product proportional to the lactulose present.

Components

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

Lactulose Hydrolysis Buffer Catalog Number MAK182A	25 mL
Hydrolysis Enzyme Mix Catalog Number MAK182B	1 vl
Lactulose Reaction Buffer Catalog Number MAK182C	25 mL
Lactulose Enzyme Mix Catalog Number MAK182D	1 vl
Lactulose Probe Catalog Number MAK182E	0.3 mL
Enhancement Solution Catalog Number MAK182F	1.5 mL
Lactulose Standard, 100 mM Catalog Number MAK182G	80 µL

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.
- Fluorescence multiwell plate reader.
- Carrez Clarification Reagent (Catalog Number MAK191 or equivalent, optional for turbid food samples)

Precautions and Disclaimer

This product is 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. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Lactulose Hydrolysis Buffer, Lactulose Reaction Buffer, Lactulose Probe, and Enhancement Solution – Allow buffers to come to room temperature before use. Store at –20 °C and use within 1 year.

Hydrolysis Enzyme Mix and Lactulose Enzyme Mix – Reconstitute each in 220 µL of water. Mix well by pipetting, verify protein dissolution, then aliquot and store at –20 °C. Use within 2 months of reconstitution. Keep on ice 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.

Lactulose Standards for Fluorometric Detection
Dilute 10 µL of the 100 mM Lactulose Standard with 990 µL of water to prepare a 1 mM Standard Solution. Dilute 100 µL of the 1 mM Standard Solution into 900 µL of water to prepare a 0.1 mM Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM Standard Solution into wells in a 96 well plate, generating 0 (blank), 200, 400, 600, 800, and 1,000 pmole/well standards. Add Lactulose Hydrolysis Buffer to each well to bring the volume to 30 µL.

Sample Preparation
Clear liquid samples can be assayed directly. Turbid liquid samples can be clarified using the Carrez Clarification Reagent. Centrifuge at 10,000 × *g* for 2 minutes. Transfer clear supernatant to a separate tube.

Solid samples (100 mg) such as foods must be homogenized in 1 mL water. Clarify 100 µL of the homogenized sample using the Carrez Clarification Reagent. Centrifuge at 10,000 × *g* for 2 minutes. Transfer clear supernatant to a separate tube.

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

Add 2–25 µL of samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 30 µL with Lactulose Hydrolysis Buffer.

Fructose in samples can result in a sample background. To correct for the fructose background, include a Sample Blank for each sample by omitting the Hydrolysis Enzyme Mix. The Sample Blank readings can then be subtracted from the sample readings.

Hydrolysis Reaction
Add 2 µL of the Hydrolysis Enzyme Mix to each well containing samples or standards. Add 2 µL water to the Sample Blank wells. Cover the plate and incubate at 37 °C for 30 minutes.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 70 µL of Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	**Samples, Blanks, and Standards**
Lactulose Buffer	65 µL
Lactulose Enzyme Mix	2 µL
Lactulose Probe	3 µL

2. Add 70 µL of the 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. Then add 15 µL of Enhancement Solution and mix.
   Note: The Enhancement Solution linearizes the response and increases the sensitivity ~8–fold.

3. Measure fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Lactulose Standard. Correct for the background by subtracting the blank standard value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Lactulose Standards to plot a 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 fluorescence measurement. Using the corrected fluorescence measurement, determine the amount of lactulose present in the sample from the standard curve.

Concentration of Lactulose

$S_a/S_v = C$

where:
S_a = Amount of Lactulose in sample well (pmole) from standard curve
S_v = Sample volume (μL) added to well
C = Concentration of Lactulose in sample

Lactulose molecular weight: 342.3 g/mole

Sample Calculation

Amount of Lactulose (S_a) = 258.4 pmole
(from standard curve)

Sample volume (S_v) = 25 μL

Concentration of Lactulose in sample:

$$258.4 \text{ pmole}/25 \text{ μL} = 10.34 \text{ pmole}/\text{μL}$$

$$10.34 \text{ pmole}/\text{μL} \times 342.3 \text{ pg/pmole} = 3539 \text{ pg}/\text{μL}$$

References

1. Weber, F.L. Jr., Lactulose and combination therapy of hepatic encephalopathy: the role of the intestinal microflora. Dig. Dis., **14**(Suppl. 1), 53–63 (1996).
2. Guerra-Ordaz, A.A. et al., Lactulose and *Lactobacillus plantarum*: a potential complementary synbiotic to control postweaning colibacillosis in piglets. Appl. Environ. Microbiol., doi: 10.1128/AEM.00770-14 (2014).

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.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use Carrez Clarification Reagent precipitation to deproteinize samples
	Solid 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 Master 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 plate well
	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