Technical Bulletin

High Sensitivity Lactulose Assay Kit

Catalog Number MAK182

Product Description

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

The 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 nm/λ_{Em} = 587 nm) product proportional to the lactulose present.

Components

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

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

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White flat-bottom 96-well plates for fluorometric assay. White plates are recommended for this assay. Cell culture or tissue culture treated plates are **not** recommended.
- Microcentrifuge capable of RCF ≥ 10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Carrez Clarification Reagent Kit (optional, for use with turbid samples) (Catalog Number MAK191 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

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

Hydrolysis Enzyme Mix and Lactulose Enzyme Mix: Reconstitute each vial with 220 µL of Lactulose Reaction Buffer and mix well. Verify that the protein is dissolved by careful visual inspection. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles. Keep the enzymes on ice while in use. Frozen enzyme solutions are stable for 2 months.

Procedure

Sample Preparation

Liquid Samples

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

Solid Samples

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

For All Samples

1. For unknown samples, it is recommended to test several sample volumes to make sure the readings are within the standard curve range.
2. Add 2–25 µL of Samples into duplicate wells of a 96-well plate.
3. Bring each Sample well to a final volume of 30 µL with Lactulose Hydrolysis Buffer.

Sample Background Control

Fructose in samples can result in sample background and can interfere with the assay. To correct for the fructose background, include a Sample Background Control for each Sample by preparing parallel Sample well(s).

Standard Curve Preparation

1. Prepare a new standard curve each time the assay is run.
2. Prepare a 1 mM Lactulose Standard by mixing 10 µL of the 100 mM Lactulose Standard with 990 µL of purified water.
3. Prepare a 0.1 mM Lactulose Standard by further diluting 100 µL of the 1 mM Lactulose Standard with 900 µL of purified water.
4. Prepare Lactulose Standards in separate wells of the 96-well plate according to Table 1.

Table 1.
Preparation of Lactulose Standards

Well	0.1 mM Lactulose Standard	Lactulose Hydrolysis Buffer	Lactulose (pmole/ well)
1	0 µL	-	0
2	2 µL	28 µL	200
3	4 µL	26 µL	400
4	6 µL	24 µL	600
5	8 µL	22 µL	800
6	10 µL	20 µL	1000

Hydrolysis Reaction

Add 2 µL of the Hydrolysis Enzyme Mix to each well containing Samples or Standards. Add 2 µL of purified water to each of the Sample Background Control wells. Cover the plate and incubate at 37 °C for 30 minutes.

Assay Reaction

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 70 µL of Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mix

Reagent	Reaction Mix
Lactulose Reaction Buffer	65 µL
Lactulose Enzyme Mix	2 µL
Lactulose Probe	3 µL

2. Add 70 µL of the Reaction Mix to each of the wells and mix well.
3. Incubate the plate for 30 minutes at 37 °C, protected from light.
4. Add 15 µL of Enhancement Solution to each well and mix thoroughly.
   Note: The Enhancement Solution linearizes the response and increases the sensitivity ~8–fold.
5. Measure the fluorescence intensity (RFU) at λ_{Ex} = 535 nm/λ_{Em} = 587 nm).

Results

1. Subtract the 0 Lactulose Blank RFU reading (Well #1) from all other Standard RFU readings.
2. Plot the Lactulose Standard Curve using the corrected Standard RFU values.
3. Subtract the Sample Background Control RFU value from each respective Sample RFU reading to obtain the corrected fluorescence measurement (ΔRFU).
4. Using the corrected fluorescence measurement (ΔRFU), determine the amount of lactulose present in the Sample (S_a)from the Lactulose Standard curve.
5. Calculate the lactulose concentration using the following equation:

   Lactulose (pmol/µL or nmol/mL or µM) =

$$\frac{S_a}{S_v}\ x\ DF$$

where

S_a = Amount of Lactulose in sample well (pmole) from standard curve

S_v = Sample volume (µL) added to well

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Lactulose in samples can also be expressed in mg/L of sample. Lactulose molecular weight: 342.3 g/mol.

Figure 1.
Time course of Standard Curve of 0-1 nmol lactulose before enhancement and signal after enhancement.

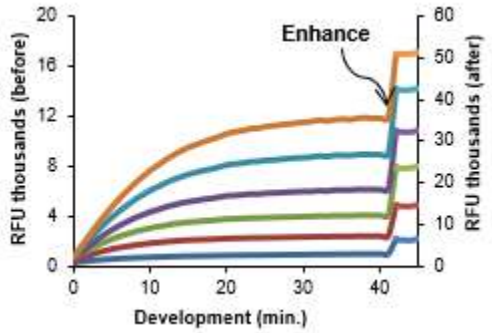

Figure 2.
Standard Curve of Lactulose before and after enhancement (Not corrected for background).

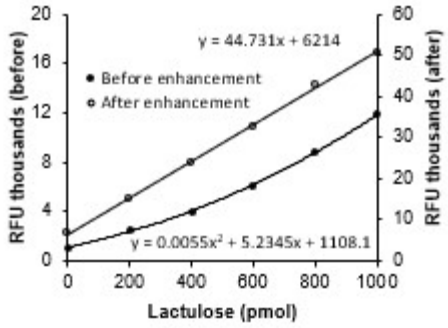

Figure 3.
Lactulose concentration results obtained for milk. 100 µL of milk were treated with the Carrez Clarification Reagent Kit, then 10 µL were diluted 1:20 with Lactulose Hydrolysis Buffer and analyzed following the protocol.

2% milk diluted 20X	RFU
10 µl	20956
blank	7173
Δ	13783
pmoles	308
µM (in milk)	678

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