EnzyChrom™ Lactulose Assay Kit (ELTL-100)

Quantitative Colorimetric Lactulose Determination

DESCRIPTION

LACTULOSE ($C_{12}H_{22}O_{11}$) is a synthetic sugar that is non-digestible by the gut. It is used to treat constipation and hyperammonemia. It may also be used for intestinal permeability test for leaky gut. Lactulose can be found in milk-based products. Raw milk normally contains extremely low or no level of lactulose. However, the lactulose level in milk will increase after heat-treated.

BioAssay Systems' lactulose assay kit is based on β-galactosidase catalyzed oxidation of lactulose, which generates D-fructose and D-galactose. The generated D-fructose reacts with the specific reagent to form a colored product whose color intensity, measured at 565 nm, is proportional to the lactulose concentration in the sample.

KEY FEATURES

Fast and sensitive. Use of 40 µL sample. Linear detection range 3 to 300 µM lactulose in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 60 minutes. Room temperature assay. No 37°C heater is needed.

High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: lactulose in food, beverage, agricultural products, and biological samples such as urine.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	6 mL	**Standard:**	400 µL 15 mM Lactulose
Enzyme A:	Dried	**Enzyme B:**	120 µL
Enzyme Buffer:	150 µL	**PMS Solution:**	1.5 mL

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Reagent Preparation: Reconstitute Enzyme A by adding 120 µL Enzyme Buffer to the Enzyme tube. Make sure enzyme is fully dissolved by pipetting up and down. Store reconstituted enzyme at -20°C and use within 1 month.

Sample Preparation: clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor *n*.

Solid samples can be homogenized in distilled water followed by filtration or centrifugation (e.g. 5-10 min 14,000 rpm). *Beverage samples* can be assayed directly. Check the pH of the sample and neutralize if necessary. *Milk samples* should be cleared by mixing 600 µL milk with 100 µL 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor *n* = 1.36)

Biological fluid samples (e.g. urine) can be assayed directly. Appropriate dilution in distilled water may be required.

Procedure using 96-well plate

1. *Standards*. Prepare 500 µL 300 µM Premix by mixing 10 µL of the Standard (15 mM) and 490 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H_2O	Lactulose (µM)
1	100 µL + 0 µL	300
2	60 µL + 40 µL	180
3	30 µL + 70 µL	90
4	0 µL + 100 µL	0

2. Transfer 40 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 40 µL of each sample into two separate wells, one serving as a sample blank well (OD_{BLANK}) and one as a sample well (OD_{SAMPLE}).
3. Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 50 µL Assay Buffer, 14 µL PMS Solution, 1 µL Enzyme A, and 1 µL Enzyme B. Prepare Blank Working Reagent (BWR) by mixing for each sample blank well, 50 µL Assay Buffer, 1 µL Enzyme A, and 14 µL PMS Solution (i.e. no Enzyme B).

   Keep WR and BWR protected from light. Add 60 µL WR to the four Standards and the Sample Wells. Add 60 µL BWR to the Sample Blank Wells. *Do not expose Working Reagent to light for more than 10 minutes. Incubate 60 min at room temperature in the dark.*
4. Read optical density at 565 nm (520-600 nm).

CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the lactulose concentration of Sample.

$$[\text{Lactulose}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

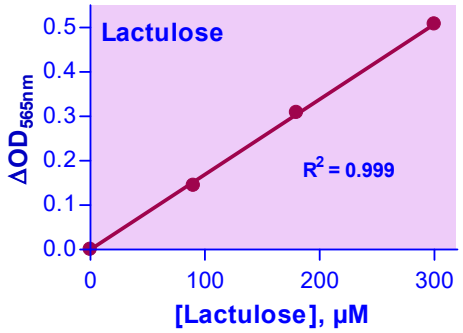
OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

Note: if the sample OD value is higher than OD for the 300 µM lactulose standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM lactulose equals 34.2 mg/dL, or 342 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate or cuvette reader.



Standard Curve in 96-well plate assay

LITERATURE

1. Amine, A., et al (2000). A new enzymatic spectrophotometric assay for the determination of lactulose in milk. Analytica Chimica Acta 406.2: 217-224.
2. Goyal, Ajesh, et al (2014). Frequency and factors associated with increased small intestinal permeability in patients with portal hypertension. Tropical Gastroenterology 34.3: 136-143.
3. Teixeira, Tatiana FS, et al (2012). Intestinal permeability parameters in obese patients are correlated with metabolic syndrome risk factors. Clinical Nutrition 31.5: 735-740.