EnzyChrom™ Lactose Assay Kit (ELAC-100)

Quantitative Colorimetric Lactose Determination

DESCRIPTION

Lactose ($C_{12}H_{22}O_{11}$), also called milk sugar, is a disaccharide that consists of β-D-galactose and α/β-D-glucose through a β1-4 glycosidic linkage. Lactose is the major sugar and makes up 2–8% of milk. Simple, direct and high-throughput assays for lactose determination find wide applications. BioAssay Systems' assay uses specific enzyme-coupled reactions in which lactose is cleaved and the resulting galactose forms a colored product. The color intensity at 570nm or fluorescence intensity at 530nm/585nm is directly proportional to the lactose concentration in the sample.

KEY FEATURES

Use as little as 20 µL samples. Linear detection range in 96-well plate: 17 to 2000 µM lactose for colorimetric assays and 6 to 100 µM for fluorimetric assays.

APPLICATIONS

Assays of lactose in milk and other biological samples.
Drug Discovery/Pharmacology: effects of drugs on lactose metabolism.
Food and Beverages: lactose in food and beverages products.

KIT CONTENTS

Assay Buffer:	10 mL	**Enzyme Mix:**	Dried
Dye Reagent:	120 µL	**Lactase:**	Dried
Standard (20 mM Lactose): 1 mL			

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

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIEMTRIC PROCEDURE

Note: (1) glycerol and SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation. (2) For samples containing galactose, a sample blank is necessary (see Procedure); (3) This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample treatment: 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).

1. Equilibrate all components to room temperature. Reconstitute the Lactase and Enzyme mix with 120 µL dH_2O. Reconstituted Lactase and Enzyme mix are stable for 3 months if stored at -20°C. During experiment, keep reconstituted Lactase and Enzyme Mix in a refrigerator or on ice.

2. *Standards and samples*: prepare 400 µL 2000 µM Standard by mixing 40 µL 20 mM standard with 360 µL dH_2O. Dilute standard in dH_2O as follows.

No	2000 µM STD + H_2O	Vol (µL)	Lactose (µM)
1	100 µL + 0 µL	100	2000
2	80 µL + 20 µL	100	1600
3	60 µL + 40 µL	100	1200
4	40 µL + 60 µL	100	800
5	30 µL + 70 µL	100	600
6	20 µL + 80 µL	100	400
7	10 µL + 90 µL	100	200
8	0 µL +100 µL	100	0

Transfer 20 µL standards and 20 µL samples into separate wells of a clear flat-bottom 96-well plate. *Note: if a sample is known to contain galactose, transfer 20* µL s*ample in duplicate* (one sample and one sample blank).

3. *Reaction*. For each reaction well, mix 85 µL Assay Buffer, 1 µL Lactase, 1 µL Enzyme Mix (vortex briefly before pipetting), and 1 µL Dye Reagent in a clean tube. (Note: for the sample blanks, prepare a control Working Reagent which is the same except WITHOUT the 1 µL Lactase). Transfer 80 µL Working Reagent into each reaction (and control) well. Tap plate to mix. Incubate 30 min at room temperature.

4. Read optical density at 570nm (550-585nm).

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 6 to 100 µM lactose. Prepare 100 µM lactose standard by mixing 5 µL 20 mM standard with 995 µL H_2O. Then dilute standards in H_2O (see *Colorimetric Procedure*) to 100, 80, 60, 40, 30, 20, 10 and 0 µM.

1. Transfer 20 µL standards and 20 µL samples into separate wells of a *black* 96-well plate. Prepare Sample Blank if necessary.

2. Add 80 µL Working Reagent, tap plate to mix. Incubate 30 min.

3. Read fluorescence at λ_{ex} = 530nm and λ_{em} = 585nm.

Notes: If the calculated lactose concentration of a sample is higher than 2000 µM in colorimetric assay or 100 µM in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor n.

CALCULATION

Subtract blank value (water, #8) from the standard values and plot the ΔOD or ΔRFU against standard concentrations. Determine the slope and calculate the lactose concentration of Sample,

$$\textit{Colorimetry:}\quad [\text{Lactose}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times n \quad (\mu M)$$

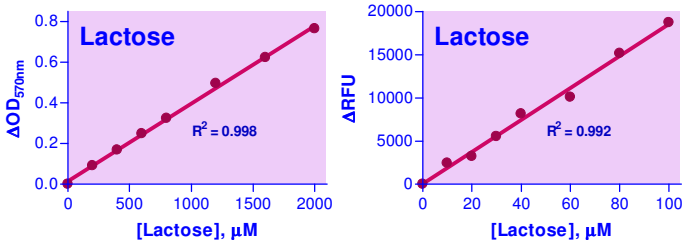
$$\textit{Fluorimetry:}\quad [\text{Lactose}] = \frac{RFU_{SAMPLE} - RFU_{BLANK}}{\text{Slope}} \times n \quad (\mu M)$$

OD_{SAMPLE}, OD_{BLANK}, RFU_{SAMPLE}, RFU_{BLANK} are optical density and fluorescence values of the Sample and Blank. The Blank is water if there is no galactose, and Sample Blank if sample contains galactose. n is the dilution factor.
Conversions: 1 mM lactose equals 34.2 mg/dL, 0.0342% or 342 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates, optical density plate reader; black 96-well plates and fluorescence plate reader.

Lactose Standard Curves



96-well colorimetric assay ***96-well fluorimetric assay***

PUBLICATIONS

1. Xue, H et al. (2020). Lactose-induced chronic diarrhea results from abnormal luminal microbial fermentation and disorder of ion transport in the colon. Frontiers in Physiology, 11, 877.

2. Snyder, N. A., Palmer, M. V., Reinhardt, T. A., & Cunningham, K. W. (2019). Milk biosynthesis requires the Golgi cation exchanger TMEM165. Journal of Biological Chemistry, 294(9), 3181-3191.

3. Vabbilisetty, P and S Xue-Long (2014). Liposome surface functionalization based on different anchoring lipids via Staudinger ligation. Organic and Biomolecular Chemistry 12(8): 1237-44.