Lactose Assay Kit (Colorimetric)

LS-K289-100 (100 Tests) • **Store at -20°C**

Introduction

Lactose ($C_{12}H_{22}O_{11}$), also called milk sugar, is a disaccharide that consists of ß-D-galactose and α/ß-D-glucose through a ß1-4 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. This 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 fluorometric 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.

Components

Component	K289-100 100 Tests
Assay Buffer	10 mL
Dye Reagent	120 µL
Enzyme Mix	Dried
Lactase	Dried
Standard (20 mM Lactose)	1 mL

Materials Not Supplied

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

Storage

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

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
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Assay Procedure

Colorimetric 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 sample 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).

Fluorometric Procedure

For fluorometric 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 fluorometric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor *n*.

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Calculations

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,

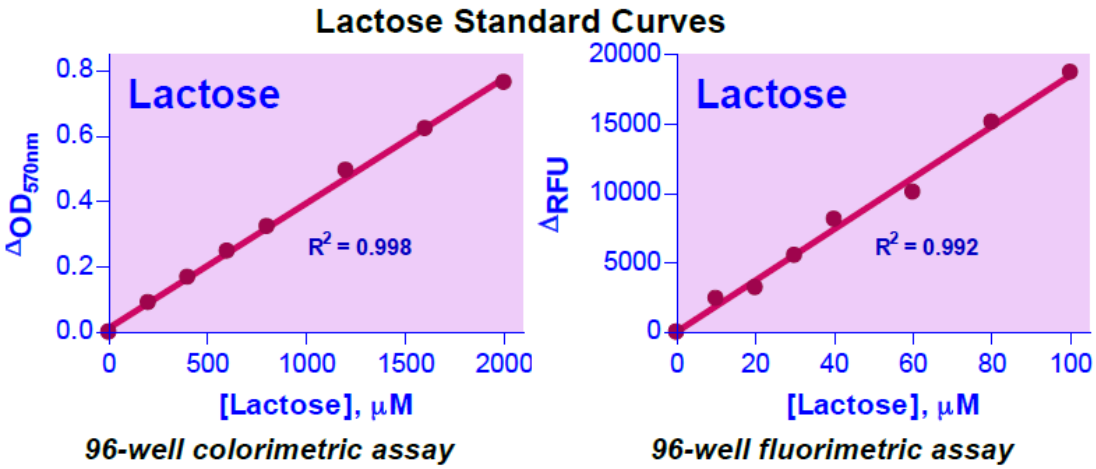
$$\textit{Colorimetry:} \quad [\text{Lactose}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times n \ (\mu M)$$

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

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.

Sample Data



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