

EnzyChrom™ Lactose Assay Kit (ELAC-100)

Quantitative Colorimetric Lactose Determination

DESCRIPTION

LACTOSE (C₁₂H₂₂O₁₁), 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:** 120 μL **Lactase:** 120 μL

Dye Reagent: 120 μL **Standard:** 1 mL 20 mM Lactose

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

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. During experiment, keep thawed 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 dH₂O. Dilute standard in dH₂O as follows.

No	2000 μM STD + H ₂ O	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. Transfer 80 μL Working Reagent into each reaction well. Tap plate to mix. Incubate 30 min at room temperature.

Note: for the sample blanks, prepare a control Working Reagent which is the same except WITHOUT the 1 μL Lactase.

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 H₂O. Then dilute standards in H₂O (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 $\lambda_{ex} = 530\text{nm}$ and $\lambda_{em} = 585\text{nm}$.

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,

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

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

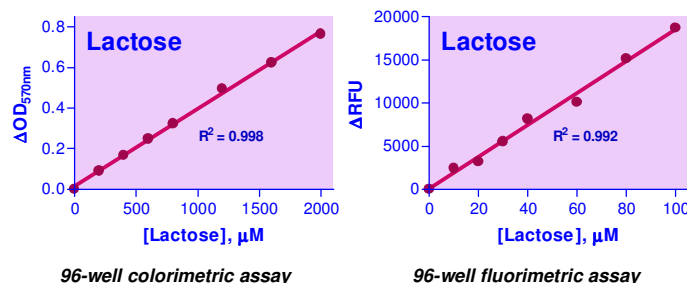
OD_{SAMPLE}, OD_{BLANK}, RFU_{SAMPLE}, RFU_{BLANK} are optical density and fluorescence values of the Sample and Blank. The Blank is water if there is no galactose, and Sample Blank if sample contains galactose. n is the dilution factor.

Conversions: 1 mM lactose equals 36 mg/dL, 0.036% or 360 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



LITERATURE

- Gülce H. et al. (2002). A novel two-enzyme amperometric electrode for lactose determination. *Anal Sci.* 18(2):147-150.
- Kleyn DH, Trout JR. (1984). Enzymatic-ultraviolet method for measuring lactose in milk: collaborative study. *J Assoc Off Anal Chem.* 67(3):637-640.
- Tsenkova R, et al (1999). Near-infrared spectroscopy for dairy management: measurement of unhomogenized milk composition. *J Dairy Sci.* 82(11): 2344-2351.