Technical Bulletin

Lactose Assay Kit

Catalogue Number MAK487

Product Description

Lactose ($C_{12}H_{22}O_{11}$) is a disaccharide comprised of galactose and glucose which form a β(1-4) glycosidic linkage. Lactose is the major sugar in milk, making up around 2–8 % of milk (by weight).

The Lactose Assay Kit uses a specific enzyme-coupled reaction in which lactose is cleaved and the resulting galactose forms a colored product. The color intensity at 570 nm or fluorescence intensity at λ_{Ex} = 530 nm/λ_{Em} = 585 nm is directly proportional to the lactose concentration in the sample.

The linear detection range of the kit is 17 - 2000 µM lactose for the colorimetric assay and 6 - 100 µM for the fluorometric assay. The kit is suitable for lactose activity determination in milk, food, beverages, and other biological samples, as well as for studying the effects of drugs on lactose metabolism.

Components

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

- Assay Buffer — 10 mL
  Catalogue Number MAK487A
- Enzyme Mix — 1 vial
  Catalogue Number MAK487B
- Dye Reagent — 120 µL
  Catalogue Number MAK487C
- Lactase — 1 vial
  Catalogue Number MAK487D
- Standard (20 mM Lactose) — 1 mL
  Catalogue Number MAK487E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes

For Use with Milk Samples

- Hydrochloric acid (Catalogue Number 320311 or equivalent)
- Sodium hydroxide (Catalogue Number 221465 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. 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.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Lactase: Reconstitute vial with 120 µL purified water. Reconstituted Lactase is stable for 3 months when stored at -20 °C. Keep reconstituted Lactase solution on ice during assay.

Enzyme Mix: Reconstitute vial with 120 µL purified water. Reconstituted Enzyme Mix is stable for 3 months when stored at -20 °C. Keep reconstituted Enzyme Mix solution on ice during assay. Briefly vortex before pipetting.

Procedure

Sample Preparation

Note: Glycerol and SH-containing reagents (for example, β–mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation.

Milk

1. Milk samples should be cleared by mixing 600 µL milk with 100 µL 6 N HCl.
2. Centrifuge for 5 minutes at 14,000 × *g*.
3. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL of 6 N NaOH.
4. The neutralized supernatant is ready for assay. Dilution factor (DF) = 1.36.

Samples Containing Galactose or Malate

For samples that contain galactose or malate, a Sample Blank is required. Transfer 20 µL of the Sample into two separate wells, one for Sample and the second for Sample Blank

All Samples

Transfer 20 µL of each Sample into separate wells of a 96-well plate.

Colorimetric Standard Curve Preparation

1. Prepare a 2000 µM Standard by mixing 40 µL of the 20 mM Standard and 360 µL of purified water.
2. Prepare Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Lactose Colorimetric Standards

Well	2000 µM Standard	Purified Water	Lactose (µM)
1	100 µL	-	2000
2	80 µL	20 µL	1600
3	60 µL	40 µL	1200
4	40 µL	60 µL	800
5	30 µL	70 µL	600
6	20 µL	80 µL	400
7	10 µL	90 µL	200
8	-	100 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a clear 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare a 100 µM Standard by mixing 5 µL of the 20 mM Standard and 995 µL of purified water.
2. Prepare Standards in 1.5 mL microcentrifuge tubes according to Table 2.

Table 2.
Preparation of Lactose Fluorometric Standards

Well	100 µM Standard	Purified Water	Lactose (µM)
1	100 µL	-	100
2	80 µL	20 µL	80
3	60 µL	40 µL	60
4	40 µL	60 µL	40
5	30 µL	70 µL	30
6	20 µL	80 µL	20
7	10 µL	90 µL	10
8	-	100 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a black 96-well plate.

Working Reagent

This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Mix enough reagent for the number of assays to be performed. Briefly vortex Enzyme Mix prior to pipetting.
   a. For each Sample and Standard well, prepare 88 µL of Working Reagent according to Table 3.
   b. For Samples requiring a Sample Blank, prepare 88 µL of Blank Working Reagent for each Sample Blank well according to Table 3.

Table 3.
Preparation of Working Reagent

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	85 µL	86 µL
Lactase	1 µL	-
Enzyme Mix	1 µL	1 µL
Dye Reagent	1 µL	1 µL

2. Transfer 80 µL of Working Reagent into each Standard and Sample well. Transfer 80 µL of Blank Working Reagent into each Sample Blank well. Tap plate to mix.

Measurement

1. Incubate the plate for 30 minutes at room temperature.
2. Measure the optical density (OD) at 570 nm or fluorescence intensity (F) at λ_{Ex} = 530 nm/ λ_{Em} = 585 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the blank reading (optical density or fluorescence intensity) of Standard #8 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD or ΔF against standard concentrations and determine the slope of the standard curve.
3. Calculate the lactose concentration of samples using the below equation:

Lactose (µM) =

$$\frac{R_{Sample} - R_{Blank}}{Slope\ (\mu M^{-1})}\ x\ DF$$

R_{Sample} = Optical density reading of Sample

R_{Blank} = Optical density reading of Blank (Standard #8 or Sample Blank)

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

Conversions: 1 mM lactose equals 34.2 mg/dL, 0.0342% or 342 ppm.

If the calculated lactose concentration of a Sample is greater than 2000 µM in the colorimetric assay or 100 µM in the fluorometric assay, dilute Sample in purified water and repeat the assay. Multiply result by the dilution factor (DF).

Figure 1.
Typical Colorimetric Lactose Standard Curve

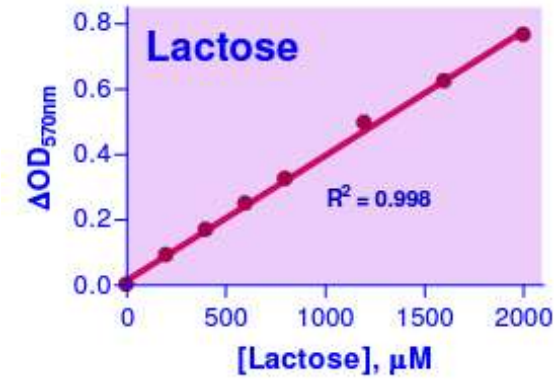
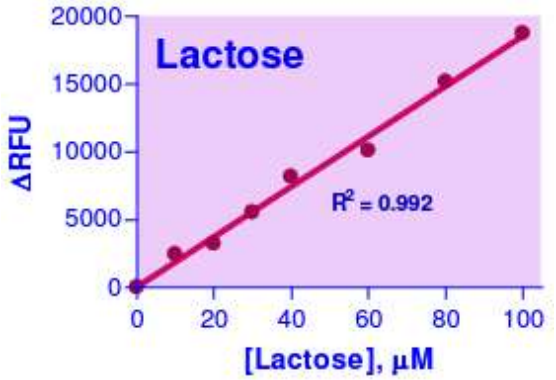


Figure 2.
Typical Fluorometric Lactose Standard Curve



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