Product Manual

Lactose Assay Kit

Catalog Number

MET-5001 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Lactose is a disaccharide form of sugar made up of one glucose and one galactose monosaccharide. In animals, millimolar levels of lactose are found in breast milk. In order for lactose to be absorbed into the bloodstream, lactase (secreted from the small intestines) cleaves lactose into glucose and galactose monosaccharides.

The inability to digest lactose is known as lactose intolerance, which arises from a reduction or loss in production of lactase, and is now more commonly known as "lactase deficiency". In most mammals, lactase deficiency is a normal condition that occurs shortly after weaning from breast milk as the primary food source. In humans, a significant population remains tolerant to lactose into adulthood due to the continued production of lactase in the small intestines. Some estimates suggest that 75% of the world's adult population is lactase deficient. Since the severity of lactose maldigestion symptoms can depend on the amount of lactose consumed, it is important to quantify the relative amounts of lactose in various dairy food sources.

Cell Biolabs' Lactose Assay Kit is a simple fluorometric assay that measures the amount of total lactose present in milk-based food products or biological samples (such as blood or urine from lactating animals) in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, lactose standards, endogenous controls*, and unknown samples. Sample lactose concentrations are determined by comparison with a known lactose standard. The kit has a detection sensitivity limit of 10 µM lactose.

Assay Principle

Cell Biolabs' Lactose Assay Kit measures total lactose within food or biological samples. First, lactase cleaves lactose into glucose and galactose. Glucose is oxidized by glucose oxidase into D-gluconic acid plus hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of lactose standard within the 96-well microtiter plate format. Samples and standards are incubated for 45 minutes and then read with a standard 96-well fluorometric plate reader (Figure 1).
**Note: Endogenous levels of glucose can interfere with the assay. Therefore, an endogenous control must be run for each sample to account for potential interference of these molecules.*

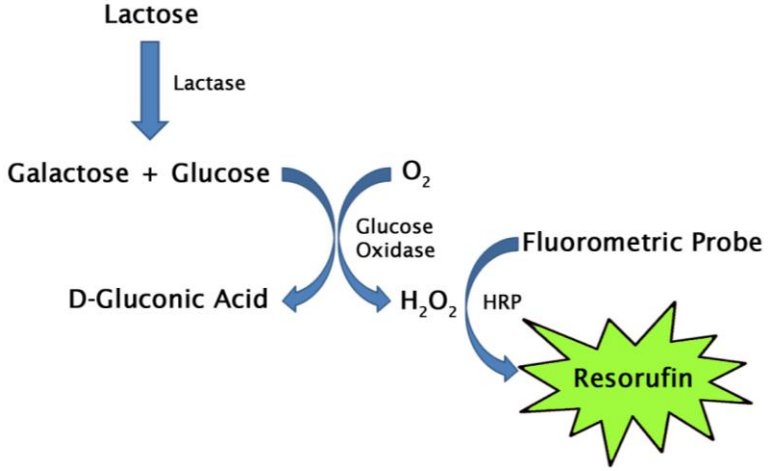

Figure 1. Lactose assay principle.

Related Products

1. STA-680: Glucose Assay Kit (Colorimetric)
2. STA-681: Glucose Assay Kit (Fluorometric)
3. MET-5012: Lactate Assay Kit (Colorimetric)
4. MET-5013: Lactate Assay Kit (Fluorometric)

Kit Components

1. Lactose Standard (Part No. 50011C): One 200 µL tube at 20 mM
2. 10X Assay Buffer (Part No. 50012A): One 25 mL bottle
3. Fluorometric Probe (Part No. 50013C): One 50 µL amber vial.
4. HRP (Part No. 234402-T): One 10 µL tube at 100 U/mL

   Note: One unit is defined as the amount of enzyme that will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 and 20ºC.
5. Lactase (Part No. 50014C): One 500 µL tube at 20U/mL

   Note: One unit will hydrolyze 1.0 µmole of lactose per minute at pH 4.5 at 30°C.
6. Glucose Oxidase (Part No. 50015C): One 100 µL tube at 200U/mL

   Note: One unit is defined as the amount of enzyme that will oxidize 1.0 micromole of beta-D-glucose to D-gluconic acid and hydrogen peroxide per minute at pH 5.1 at 35°C.

Materials Not Supplied

1. Deionized water
2. 1X PBS
3. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate

Storage

Store the 10X Assay Buffer at room temperature. Store all other components at -20ºC. The Fluorometric Probe is light sensitive and must be protected accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, Lactase 1:10 and Glucose Oxidase 1:50 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, 100 µL of Lactase and 20 µL of Glucose Oxidase to 868 µL 1X Assay Buffer for a total of 1 mL. The above example Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.
  Note: Scale the described example up or down as needed and prepare only enough for immediate use.

- Endogenous Control Mix: Prepare Endogenous Control Mix (no Lactase) by diluting the Fluorometric Probe 1:100, HRP 1:500, and Glucose Oxidase 1:50 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, 20 µL of Glucose Oxidase, to 968 µL 1X Assay Buffer for a total of 1 mL. The above example is enough for 20 assays. The Endogenous Control Mix is stable for 1 day at 4ºC.
  Note: Scale the described example up or down as needed and prepare only enough for immediate use.

Preparation of Samples

- Cell culture supernatants: Cell culture media formulated with lactose or glucose should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media may be assayed directly or diluted as necessary. Prepare the Lactose standard curve in non-conditioned media without lactose or glucose.
  Note: Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Dilute the supernatant from 1:50 to 1:200 with 1X Assay Buffer just prior to performing the assay.

  Notes:
  - *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
  - *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 5).*
  - *Avoid samples containing DTT or β-mercaptoethanol since Resorufin is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

1. Prepare fresh Lactose standards before use by diluting in 1X Assay Buffer. First, dilute the Lactose Standard 20 mM stock solution 1:20 in 1X Assay Buffer to make a 1 mM Lactose Solution (e.g., add 25 µL of the stock 20 mM Lactose Standard to 475 µL of 1X Assay Buffer). Vortex thoroughly.
2. Use the 1 mM Lactose Solution to prepare a series of the remaining Lactose standards according to Table 1.

Standard Tubes	1 mM Lactose Solution (µL)	1X Assay Buffer (µL)	Lactose (µM)	Lactose (mg/dL)
1	80	320	200	6.84
2	64	336	160	5.47
3	48	352	120	4.10
4	32	368	80	2.74
5	16	384	40	1.37
6	8	392	20	0.68
7	4	396	10	0.34
8	0	400	0	0

Table 1. Preparation of Lactose Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one positive well and one background control well.
2. Add 50 µL of each standard into wells of a black microtiter plate suitable for a fluorescence plate reader.
3. Add 50 µL of each unknown sample to each of two separate wells.
4. Add 50 µL of Reaction Mix to all standard wells and one half of the paired sample wells.
5. Add 50 µL of Endogenous Control Mix to the remaining paired sample wells.
6. Mix all well contents thoroughly and incubate for 45-60 minutes at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
7. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
8. For each unknown sample, subtract the RFU reading using the Endogenous Control Mix from the RFU reading using the Reaction Mix.
9. Calculate the concentration of lactose within samples by comparing the sample RFU to the standard curve.

Example of Results

The following figures demonstrate typical Lactose Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

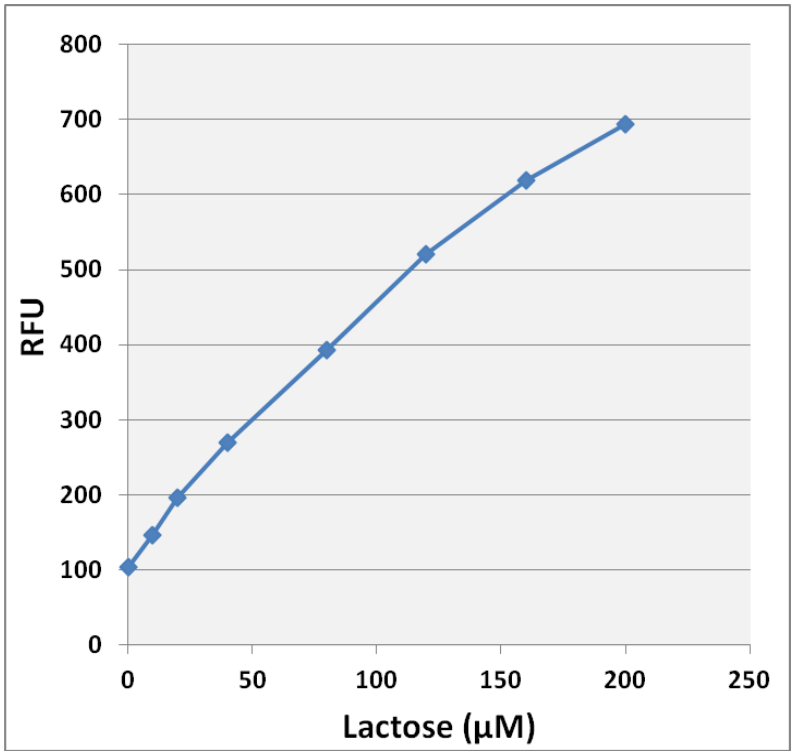

Figure 2: Lactose standard curve.

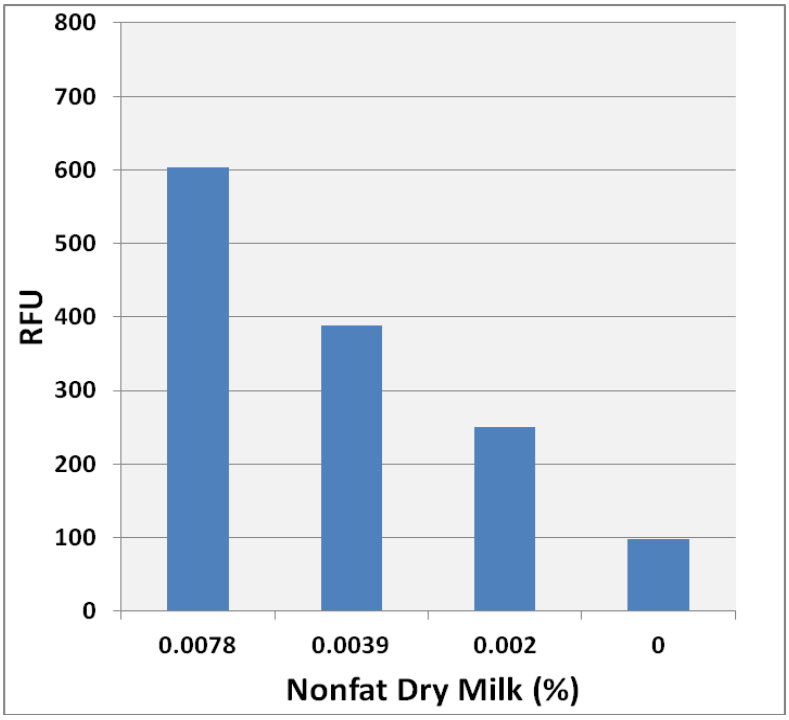

Figure 3: Lactose detection in bovine nonfat dry milk using the Lactose Assay Kit.

References

1. Swallow DM (2003). *Annual Review of Genetics* **37**: 197–219.
2. Ingram CJ, Mulcare CA, Itan Y, Thomas MG, Swallow DM (Jan 2009). *Hum Genet* **124** (6): 579-91.
3. Lactose Tolerance and Milk Consumption. (1988) *Am J Clin Nutr.* Ch 3 Lactose content of milk and milk products, **48**:1099-1104.
4. Argüelles AF, Rodríguez L, Tenias J, Otero M, Casellas Jordá F, Blay Cortés G, Lucendo Villarín AJ, Domínguez Jiménez JL, Carballo Alvarez F, Aranoff SL, Berkowitz K, Shreiner B, and Want L (2015) *Rev Esp Enferm Dig.* **107.**
5. Votyakova TV, and Reynolds IJ (2001) *Neurochem*. **79**:266.

Recent Product Citation

1. Li, Y. et al. (2021). Nrf2-ARE Signaling Partially Attenuates Lipopolysaccharide-Induced Mammary Lesions via Regulation of Oxidative and Organelle Stresses but Not Inflammatory Response in Mice. *Oxid Med Cell Longev*. doi: 10.1155/2021/8821833.

Warranty

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Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com