Lactose Assay Kit

Lactose Assay Kit can be used to measure Lactose in milk, food and other biological samples.

Catalog number: ARG82174

Package: 100 tests

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

Lactose is a disaccharide. It is a sugar composed of galactose and glucose subunits and has the molecular formula C12H22O11. Lactose makes up around 2–8% of milk (by weight). The name comes from lac (gen. lactis), the Latin word for milk, plus the suffix -ose used to name sugars. The compound is a white, water-soluble, non-hygroscopic solid with a mildly sweet taste. It is used in the food industry. Lactose composes about 2–8% of milk by weight. Several million tons are produced annually as a by-product of the dairy industry.

Whey or milk plasma is the liquid remaining after milk is curdled and strained, for example in the production of cheese. Whey is made up of 6.5% solids, of which 4.8% is lactose, which is purified by crystallisation. Industrially, lactose is produced from whey permeate – that is whey filtrated for all major proteins. The protein fraction is used in infant nutrition and sports nutrition while the permeate can be evaporated to 60–65% solids and crystallized while cooling. Lactose can also be isolated by dilution of whey with ethanol.

Dairy products such as yogurt and cheese contain very little lactose, as the bacteria used to make them consume lactose during the manufacturing process. [Wikipedia: Lactose]

PRINCIPLE OF THE ASSAY

This Lactose Assay Kit provides a simple and direct procedure for measuring Lactose concentration in samples. In this assay, lactose is cleaved by lactase into glucose and galactose. The resulting galactose is then oxidized to generate a product that produces color (OD 570 nm) and fluorescence (Ex/Em 530/585 nm) with dye reagent. The color intensity at 570 nm or fluorescence intensity at λ ex/em = 530/585 nm is directly proportional to the Lactose concentration in the sample. The concentration of Lactose in the sample is then determined by comparing the signals of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	10 ml (Ready to use)	-20°C
Lactose Standard (20 mM)	1 ml	-20°C
Enzyme Mixture	1 vial (Lyophilized)	-20°C
Lactase	1 vial (Lyophilized)	-20°C
Dye Reagent	120 µl (Ready to use)	-20°C

The kit is shipped on blue-ice. Upon received, store all components at -20°C in dark. Shelf life of six months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 570 nm (550-585 nm). Or fluorescence Microplate Reader capable of measuring fluorescence at λ ex/em = 530/585 nm.
- Flat bottomed 96-well microplate for colorimetric procedure or black flat bottomed 96-well microplate for fluorimetric procedure
- Pipettes and pipette tips
- Deionized or distilled water.

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The kit is shipped on blue-ice. Upon received, store all components at -20°C in dark. Shelf life of six months after receipt.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- All materials should be equilibrated to room temperature (RT) before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- 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 pipettes is recommended.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Note:

(1) Glycerol and SH-containing reagents e.g. beta–mercaptoethanol (2-ME, beta-ME), dithiothreitol (DTT) 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 Assay Procedure section)

(3) This assay is based on a kinetic reaction.

(4) Add cold PBS in solid samples, sonicate or homogenize the samples and centrifuge at 10000 x g for 10 minutes at 4°C. Collect the supernatant for assay.

Milk Sample treatment:

Milk samples should be cleared by mixing 600 µL of milk with 100 µL of 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL of 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor **N** = 1.36).

Original milk samples can be stored at –20°C to –80°C for up to 90 days.

Dilution:

- If the initial assay found samples contain Lactose higher than 2000 µM for the colorimetric assay, or 100 µM for the fluorimetric assay, the samples can be diluted with distilled water and then re-assay the samples. For the calculation of the concentrations this dilution factor (N) has to be taken into account. The sample must be well mixed with the diluents buffer before assay. (It is recommended to do pre-test to determine the suitable dilution factor).

REAGENT PREPARATION

- **Enzyme Mixture:** Equilibrate Enzyme Mixture to room temperature. Dissolve the Enzyme Mixture in **120 µl** of **distilled water.** Pipette up and down to mix well, and make sure the Enzyme Mixture is fully dissolved completely before assay. The reconstituted Enzyme Mixture is stable for 3 months when it stores at-20°C. Before each use of the Enzyme Mixture, pipette up and down or brief mix to assure the enzyme is mixed well. During experiment, keep reconstituted Enzyme Mixture in a refrigerator or on ice.
- **Lactase:** Equilibrate Lactase to room temperature. Dissolve the Lactase in **120 µl** of **distilled water**. Pipette up and down to mix well, and make sure the Lactase is fully dissolved completely before assay. The reconstituted Lactase is stable for 3 months when it stores at -20°C. Before each use of the Lactase, pipette up and down or brief mix to assure the enzyme is mixed well. During experiment, keep reconstituted Lactase in a refrigerator or on ice.
- **Standard:**

  For Colorimetric Procedure:

  - Standard Stock: Mixing **40 µL** of **20 mM Standard stock** and **360 µL deionized water** to yield 400 µL of **2000 µM Lactose** standard. Dilute the 2000 µM Lactose standard with deionized water to yield standard concentration as 2000 µM, 1600 µM, 1200 µM, 800 µM, 600 µM, 400 µM, 200 µM and 0 µM (Deionized water only).

Dilute standard as follows.

Standard No.	Standard Conc. µM	Deionized water (µl)	2000 µM Standard stock (µl)
S1	2000	0	100 µl
S2	1600	20	80 µl
S3	1200	40	60 µl
S4	800	60	40 µl
S5	600	70	30 µl
S6	400	80	20 µl
S7	200	90	10 µl
S0	0	100	0

For Fluorimetric Procedure:

- For fluorimetric assays, the linear detection range is 6 to 100 µM Lactose.
- Mixing **5 µL** of **20 mM Standard stock** and **995 µL** of **deionized water** to yield 1000 µL of **100 µM Lactose**.
- Dilute the 100 µM Lactose standard with deionized water to yield standard concentration as 100 µM, 80 µM, 60 µM, 40 µM, 30 µM, 20 µM, 10 µM and 0 µM (Deionized water only).

Standard No.	Standard Conc. µM	Deionized water (µl)	100 µM Standard stock (µl)
S1	100	0	100 µl
S2	80	20	80 µl
S3	60	40	60 µl
S4	40	60	40 µl
S5	30	70	30 µl
S6	20	80	20 µl
S7	10	90	10 µl
S0	0	100	0

- **Working Reagent:**

  For each reaction combine the following (*Prepare before use)*:

  85 µL of Assay Buffer

  1 µL of Enzyme Mixture (vortex briefly before using)

  1 µL of Lactase

  1 µL Dye Reagent.

  Mix well in a clean tube. Transfer 80 µl of Working Reagent to each sample and standard wells.

- **Sample Blank Reagent:**

  If a sample is known to contain galactose, it is recommended to add Sample Blank assay to eliminate the endogenous galactose signal. For each Sample Blank reaction combine the following (*Prepare before use)*:

  85 µL of Assay Buffer

  1 µL of Enzyme Mixture (vortex briefly before using)

  1 µL Dye Reagent.

  1 µl of distilled water

  Note: There is **NO** Lactase in Sample Blank Reagent. Mix well in a clean tube. Transfer 80 µl of Sample Blank Reagent to each Sample Blank well.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use. During experiment, keep reconstituted Enzyme Mixture and Lactase in a refrigerator or on ice. If a sample is known to contain galactose, including sample blank assay is recommended.

For Colorimetric Procedure:

1. Add **20 µl** of **each sample and standard** in flat bottomed 96 well plate.
   (Optional) When sample blank assay is necessary (sample is known to contain galactose). Add 20 µl of each standard in flat bottomed 96 well plate. Add 20 µl of each sample in two separate wells of the plate. Well 1 for sample assay and well 2 for sample blank.
2. Add **80 µL** of the **Working Reagent** to each sample and standard well.
3. Add **80 µL** of the **Sample Blank Reagent** to each Sample Blank well.
4. Gently tap the plate to ensure thorough mixing. Incubate for **30 min at room temperature in dark.**
5. Read the OD with a microplate reader at **570 nm (550 - 585nm)** immediately.

For Fluorimetric Procedure (black 96 well plate is used):

1. Add **20 µl** of **each sample and standard** in **black 96 well plate**.
   *(Optional) When sample blank assay is necessary (sample is known to contain galactose). Add 20 µl of each standard in **black 96 well plate**. Add 20 µl of each sample in two separate wells of the **black 96 well plate**. Well*

1 for sample assay and well 2 for sample blank assay.

2. Add **80 µL** of the **Working Reagent** to each sample and standard well.
3. Add **80 µL** of the **Sample Blank Reagent** to each Sample Blank well.
4. Gently tap the plate to ensure thorough mixing. Incubate for **30 min at room temperature in dark**.
5. Read fluorescence intensity at **λ ex = 530 nm and λ em = 585 nm** immediately.

Summary:

A. For Colorimetric Procedure:

	Assayed sample	Sample Blank	Standards
Sample	20 µl	20 µl	-
Standards	-	-	20 µl
Working Reagent	80 µl	-	80 µl
Sample Blank Reagent	-	80 µl	-
Mix well and incubate for 30 min at RT in dark.			
Read the OD with a microplate reader at 570 nm immediately.			

B. For Fluorimetric Procedure:

	Assayed sample	Sample Blank	Standards
Sample	20 µl	20 µl	-
Standards	-	-	20 µl
Working Reagent	80 µl	-	80 µl
Sample Blank Reagent	-	80 µl	-
Mix well and incubate for 30 min at RT in dark.			
Read fluorescence intensity at λ ex/em = 530 / 585 nm immediately.)			

CALCULATION OF RESULTS

1. Subtract the blank value (S0) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the Lactose concentration of the Samples as follows:

For Colorimetric Method:

No sample blank assay

[Lactose] (μM)=

$N \times [(OD_{Sample} - OD_{Blank}) / Slope\ (\mu M^{-1})]$

Sample blank is included

[Lactose] (μM)=

$N \times [(OD_{Sample} - OD_{Sample\ Blank}) / Slope\ (\mu M^{-1})]$

Note:

OD_{Sample} : OD value of Assayed sample well

$OD_{Standard}$: OD value of Standards

Slope: Slope calculated from $OD_{Standards}$

OD_{Blank} : OD value of Blank well (S0)

$OD_{Sample\ Blank}$: OD value of each sample blank well.

N = dilution factor (if sample has been diluted before assay. For cleared milk the N=1.36, please refer the sample preparation section for detail)

2. For Fluorimetric Method:

No sample blank assay

[Lactose] (µM)=

$$N \times [(F_{Sample} - F_{Blank}) / Slope\ (\mu M^{-1})]$$

Sample blank is included

[Lactose] (µM)=

$$N \times [(F_{Sample} - F_{Sample\ Blank}) / Slope\ (\mu M^{-1})]$$

Note:

F_{Sample} : F value of Assayed sample well

$F_{Standard}$: F value of Standards

Slope: Slope calculated from $F_{Standards}$

F_{Blank} : F value of Blank well (S0)

$F_{Sample\ Blank}$: F value of each sample blank well

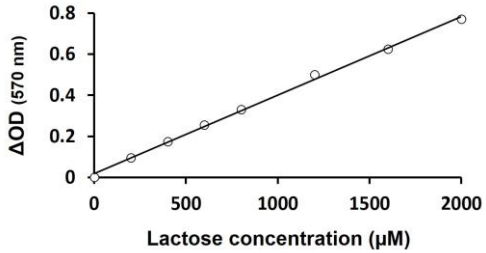
N = dilution factor (if sample has been diluted before assay. For cleared milk the N=1.36, please refer the sample preparation section for detail)

3. If the calculated Lactose concentration is > 2000 µM for the colorimetric assay, or > 100 µM for the fluorimetric assay, dilute sample in deionized water and repeat assay. Multiply result by the dilution factor N.
4. Conversions: 1 mM Lactose equals 34.2 mg/dL, 0.0342% or 342 ppm (Lactose molecular weight: 342.3)

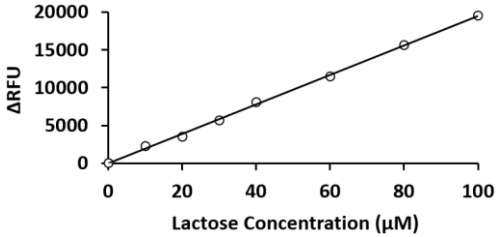
EXAMPLE OF ASSAY

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

For colorimetric assay



For fluorimetric assay



QUALITY ASSURANCE

Sensitivity

Linear detection range:

Colorimetric assays: 17 to 2000 µM

Fluorimetric assays: 6 to 100 µM

The minimum detectable dose (MDD) of Lactose was:

Colorimetric assays: 17 µM

Fluorimetric assays: 6 µM