

ab83384

Lactose Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Lactose levels in various samples

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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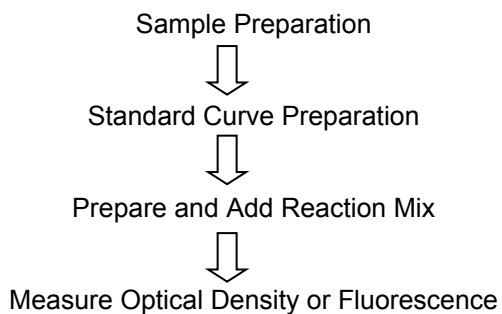
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1. Overview

Lactose ($C_{12}H_{22}O_{11}$ FW: 342.3) is an important naturally occurred disaccharide, consisting of one galactose and one glucose. Milk contains ~2-8% lactose. Some people, particularly infants, lack the enzyme necessary to digest galactose leading to galactose accumulation in blood (Galactosemia) causing enlarged liver, renal failure, cataracts and brain damage.

In Abcam's Lactose Assay Kit, Lactose is hydrolyzed to glucose and galactose. The galactose is subsequently oxidized generating color (OD_{570nm}) and fluorescence (Ex/Em 535/587 nm). Free galactose can be corrected by a background control in the absence of lactase. The Lactose Assay Kit provides a simple, convenient, and sensitive means for direct measurement of lactose levels in various biological samples (body fluids, food, growth media, etc.). Pretreatment of samples is not required. The kit can be used as a high throughput assay.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer II/Lactose Assay Buffer	25 mL
OxiRed Probe/Probe (DMSO)	0.2 mL
Lactase Enzyme/Lactase (Lyophilized)	1 vial
Galactose Enzyme Mix/Lactose Enzyme Mix (Lyophilized)	1 vial
Developer Solution V/HRP (Lyophilized)	1 vial
Lactose Standard (100 nmol/ μ l)	100 μ L

* Store kit at -20°C .

OxiRed Probe/PROBE: Ready to use as supplied. Allow to warm to room temperature to thaw the DMSO solution before use. Store at -20°C , protect from light and moisture. Use within two months.

Lactase Enzyme/LACTASE: Dissolve in 220 μ l Assay Buffer II/Lactose Assay Buffer. Aliquot and store at -20°C . Use within two months.

Galactose Enzyme Mix/LACTOSE ENZYME MIX: Dissolve in 220 μ l Assay Buffer II/Lactose Assay Buffer. Pipette up and down to completely dissolve. Store at -20°C . Use within two months.

Developer Solution V/HRP: Dissolve in 220 μ l Assay Buffer II/Lactose Assay Buffer. Aliquot and store at -20°C . Use within two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Samples (1-50 μl) can be directly added to the wells, then adjust the total volume to 50 μl with Assay Buffer II/Lactose Assay Buffer.

For unknown samples, we suggest testing several doses to make sure the readings are within the standard curve linear range.

2. Standard Curve Preparation:

a. For the colorimetric assay:

Dilute the Lactose Standard to 1 nmol/ μl by adding 10 μl of the 100nmol/ μl Lactose Standard to 990 μl of Assay Buffer II/Lactose Assay Buffer and mix well. Add 0, 2, 4, 6, 8, 10 μl into a series of wells of a 96 well plate. Adjust the volume to 50 μl /well with Assay Buffer II/Lactose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Lactose Standard.

b. For the fluorometric assay:

Dilute the Lactose Standard solution to 0.1 nmol/ μl by adding 10 μl of the Lactose Standard to 990 μl of Assay Buffer II/Lactose Assay Buffer and mix well. Then take 20 μl into 180 μl of Assay Buffer II/Lactose Assay Buffer and

mix well. Add 0, 2, 4, 6, 8, 10 μl into a series of wells of a 96 well plate. Adjust volume to 50 μl /well with Assay Buffer II/Lactose Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Lactose Standard.

The fluorometric assay is ~10 times more sensitive than the colorimetric assay.

3. Add 2 μl of Lactase Enzyme/Lactase* into each standard and sample to convert lactose to galactose.

***Note:** Free galactose interferes with the assay. If galactose is present in your samples, prepare two wells for each sample. Add 2 μl of Lactase Enzyme/Lactase to one well, add 2 μl of Assay Buffer II/assay buffer to the other well as galactose background control. Galactose background can be subtracted from the lactose assays.

4. **Lactose Reaction Mix:**

Mix enough reagent for the number of assays to be performed. For each well, prepare a total 50 μl Reaction Mix containing the following components:

Assay Buffer II/Lactose Assay Buffer	44 μl
OxiRed Probe/Probe*	2 μl
Galactose Enzyme Mix/Lactose Enzyme Mix	2 μl
Developer Solution V/HRP	2 μl

Mix well. Add 50 μl of the Reaction Mix to each well containing the Lactose Standard and test samples. Mix well. Incubate the reaction for 60 min at 37°C, protect from light.

***Note:** Using 0.4 μl of the OxiRed Probe/probe for each standard and sample in the fluorometric assay can decrease the fluorescence background significantly and thus increase detection sensitivity.

5. Measure $\text{OD}_{570\text{nm}}$ for the colorimetric assay or $\text{Ex/Em} = 535/590 \text{ nm}$ for the fluorometric assay in a microplate reader.

5. Data Analysis

Correct background by subtracting the value derived from the zero lactose control from all sample readings. The background reading can be significant and must be subtracted from sample readings.

Plot the standard curve as lactose amount (nmol) vs readings. Apply sample readings to the standard curve.

Calculate Lactose concentration:

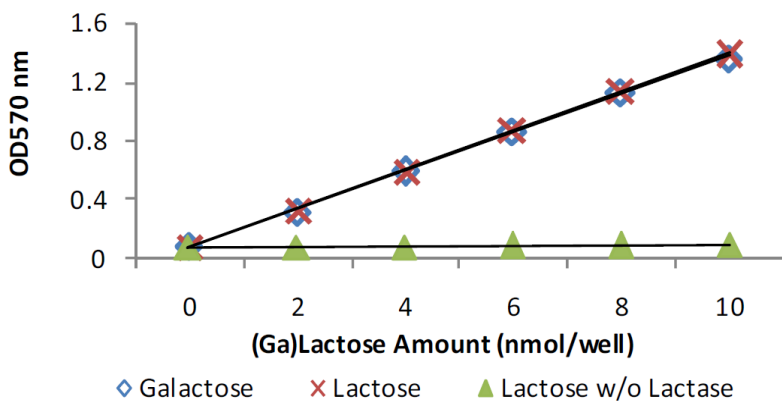
$$\text{Concentration} = \text{Ga} / \text{Sv} \text{ (nmol}/\mu\text{l or mM)}$$

Where:

Ga: Galactose amount in the sample wells (in nmol).

Sv: Sample volume added into the wells (in μl).

Lactose molecular weight: 342.3



Lactose Standard Curve. Assays were performed following the kit instructions. The kit detected galactose and lactose equally. In the absence of Lactase Enzyme/Lactase, the kit detected galactose, but not lactose.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the 10kDa spin column (ab93349)
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).

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