

Product Information

Lactose Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2129-100 100 assays	Cap Color	Part Number
Lactose Assay Buffer	25 ml	WM	K2129-C-1
Probe (DMSO solution)	0.2 ml	Red	K2129-C-2
Lactase (Lyophilized)	1 Vial	Blue	K2129-C-3
Lactose Enzyme Mix (Lyophilized)	1 Vial	Green	K2129-C-4
HRP (Lyophilized)	1 Vial	Purple	K2129-C-5
Lactose Standard (100 nmol/μl)	100 μl	Yellow	K2129-C-6

II. Introduction:

Lactose (C₁₂H₂₂O₁₁) is a disaccharide sugar consisting of one galactose and one glucose. Lactose makes up around 2 - 8% of milk. Some people, particularly infants, lack lactase to digest lactose leading to lactose accumulation in blood (Galactosemia), which then causes renal failure, enlarged liver, cataracts and brain damage.

The Lactose Colorimetric/Fluorometric Assay Kit provides a sensitive, fast and convenient way for detection of lactose levels in various biological samples (plasma, serum, other body fluids, growth media, food, etc.) based on colorimetric and fluorometric method. In the assay, lactose is hydrolyzed to glucose and galactose, which is then oxidized producing fluorescence (Ex/Em 535/587 nm) and color (O.D 570 nm). Free galactose can be corrected by a background control in the absence of lactase. The assay is performed without pretreatment of samples. The kit is suited for a high throughput assay.

III. Storage and Handling:

Store kit at -20°C, protect from light. Briefly centrifuge vials prior to opening. Allow assay buffer warm to room temperature before use, but keep enzymes on ice during the assay.

IV. Reagent Preparation:

Probe: Ready to use as supplied. Allow to come to room temperature prior to use. Store at -20°C, protect from light and moisture. Use within two months.

Lactase Dissolve in 220 μl Lactose Assay Buffer. Aliquot and store at -20°C. Use within two months.

Enzyme Mix: Dissolve in 220 μl Lactose Assay Buffer. Aliquot and store at -20°C. Use within two months.

HRP: Dissolve in 220 μl Lactose Assay Buffer. Aliquot and store at -20°C. Use within two months.

V. Lactose Assay Protocol:

1. Standard Curve Preparation:

For the colorimetric assay, dilute the Lactose Standard to 1 nmol/μl by adding 10 μl of the 100 nmol/μl Lactose Standard to 990 μl of 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 Lactose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Lactose Standard.

For the fluorometric assay, dilute the Lactose Standard solution to 0.1 nmol/ μ l by adding 10 μ l of the Lactose Standard to 990 μ l of Lactose Assay Buffer and mix well. Then take 20 μ l into 180 μ l of Lactose Assay Buffer and mix well. Add 0, 2, 4, 6, 8, 10 μ l into a series of wells of a 96 well plate. Adjust volume to 50 μ l/well with 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.

2. Sample Preparation: Sample (1 - 50 μ l) can be directly added to the wells, then adjust the total volume to 50 μ l with Lactose Assay Buffer. For unknown samples, we suggest testing several doses to make sure the readings are within the standard curve linear range.

3. Add 2 μ l of 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 μ l of Lactase to one well, add 2 μ l of 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 reagents for the number of assays to be performed. For each well, prepare a total 50 μ l Reaction Mix containing:

Lactose Assay Buffer	44 μ l
Probe	2 μ l
Lactose Enzyme Mix	2 μ l
HRP	2 μ l

Notes: The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Using 0.4 μ l of the probe for each standard and sample in the fluorometric assay can decrease the fluorescence background significantly and thus increase detection sensitivity.

5. Mix well. Add 50 μ l of the Reaction Mix to each well containing the Lactose Standard and test samples. Mix well.

6. Incubate the reaction for 60 min at 37°C, protect from light.

7. Measure OD 570 nm for the colorimetric assay or Ex/Em = 535/590 nm for the fluorometric assay in a microplate reader.

8. Calculations: Correct background by subtracting the value of the 0 lactose control from all readings. Plot standard curve as lactose amount (nmol) vs readings. Apply sample readings to the standard curve. Calculate Lactose concentration:

$$C = Ga/Sv \text{ nmol}/\mu\text{l or } \mu\text{mol}/\text{ml or mM}$$

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

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

Lactose molecular weight: 342.3.

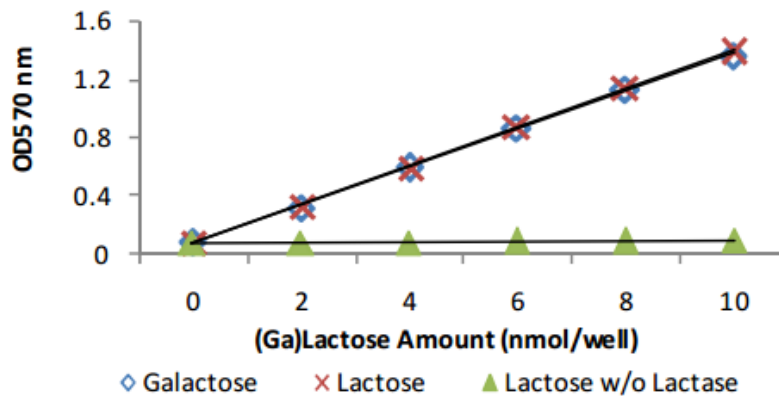


Figure Legend: Lactose Standard Curve. Assays were performed following the kit instructions. The kit detected galactose and lactose equally. In the absence of Lactase, the kit detected galactose, but not lactose.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.



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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit <http://www.apexbt.com/> or contact our technical team.

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