Amplite® Lactose Quantitation Kit

Catalog number: 40008
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 40008)
Component A: Amplite® Red	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)
Component C: Horseradish Peroxidase	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: Enzyme Mix	Freeze (< -15 °C), Minimize light exposure	1 vial
Component E: DMSO	Refrigerated (2-8 °C)	1 vial
Component F: Lactose Standard	Freeze (< -15 °C), Minimize light exposure	1 vial

OVERVIEW

Lactose, a natural disaccharide formed by the condensation of galactose and glucose molecules, is a major sugar in the milk of most species, typically ranging from 2-8%. Following ingestion, the enzyme lactase hydrolyzes lactose into its constituent monosaccharides for absorption. In specific individuals, particularly infants, the absence of the necessary galactose-digesting enzyme leads to Galactosemia, a disorder characterized by symptoms such as enlarged liver, renal failure, cataracts, and brain damage. The Amplite® Lactose Quantitation Kit employs an enzymatic assay to determine lactose concentration, converting lactose into galactose and glucose. Subsequent oxidation of galactose yields a colorimetric (570 nm) and fluorometric (Ex/Em = 540/590 nm) product, directly proportional to the initial lactose level. This versatile kit enables lactose measurement in diverse biological samples, including serum, plasma, other body fluids, food, and growth media. For researchers and clinicians, the kit proves invaluable, facilitating the assessment of lactose metabolism and lactose-related health implications across different biological contexts.

AT A GLANCE

Important

Thaw all the kit components to room temperature before starting the experiment.

Protocol Summary

1. Prepare and add Lactose Standards and test samples (50 µL)
2. Prepare and add Enzyme-dye assay reaction mixture (50 µL)
3. Incubate at 37 °C for 10 - 30 minutes
4. Monitor Fluorescence intensity at Ex/Em = 540/590 nm or absorbance at OD = 570 nm

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	570 nm
Emission	590 nm
Excitation	540 nm
Recommended plate	Solid black

Absorbance microplate reader

Absorbance	570 nm
Recommended plate	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Amplite® Red Stock Solution (200X)

1. Prepare stock solution by adding 25 µL of DMSO (Component E) into the vial of Amplite® Red (Component A). The stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20 °C.

   Note: Amplite® Red is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM.

   Note: Amplite® Red is unstable at high pH (>8.5). The reaction should be performed at pH 7 – 8. The provided assay buffer (pH 7.4) is recommended.

HRP Stock Solution (50X)

1. Add 1 mL of assay buffer (Component B) into the vial of horseradish peroxidase (Component C).

   Note: The unused HRP solution should be divided into single-use aliquots and stored at -20 °C.

Lactose Stock Solution

1. Add 500 µL of assay buffer (Component B) into the vial of Lactose Standard (Component F) to make a 20 mM stock solution. Diluted to 1 mM in assay buffer (10 uL of 20 mM to 190 uL assay buffer).

   Note: The unused Lactose Standard stock solution should be stored at -20 °C.

Enzyme Mix Stock Solution (50X)

1. Add 100 µL of assay buffer (Component B) into the vial of enzyme mix (Component D).

   Note: The unused enzyme mix stock solution should be divided into single-use aliquots and stored at -20 °C.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/40008

Lactose Standard for Fluorometric Analysis

Add 12.5 µL of a diluted Lactose Standard stock solution (1 mM) to 237.5 µL of Assay Buffer (Component B) to make a 50 µM (LS1) dilution. Then perform 1:2 serial dilutions to get serially diluted Standard (LS2 – LS7).

PREPARATION OF WORKING SOLUTION

Prepare the enzyme-dye assay reaction mixture according to the following table, protected from light.

Table 1. Assay reaction mixture for a 96-well plate (2X)

Components	Volume
Amplite® Red Stock Solution	25 µL
HRP Stock Solution	100 µL
Enzyme Mix	100 µL
Assay Buffer (Component B)	4.75 mL
Total Volume	5 mL

SAMPLE EXPERIMENTAL PROTOCOL

Table 2. Layout of Lactose Standards and test samples in a solid black 96-well microplate.

LS=Lactose Standards (LS1 - LS7, 200 to 3.12 µM for colorimetric analysis and 50 to 0.78 µM for fluorometric analysis); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
LS1	LS1	...	...
LS2	LS2	...	...
LS3	LS3		
LS4	LS4		
LS5	LS5		
LS6	LS6		
LS7	LS7		

Table 3. Reagent composition for each well.

Well	Volume	Reagent
LS1-LS7	50 µL	Serial dilutions (according to Table 2)
BL	50 µL	Assay Buffer
TS	50 µL	Sample

Sample Protocol

1. Prepare Lactose Standards (LS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of Enzyme-dye reaction mixture to each well of Sodium Standards, blank control, and test samples to make the assay volume 100 µL/well. For a 384-well plate, add 25 µL into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 5 to 10 minutes, protected from light.
4. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm (cut off at 570 nm) or absorbance with an absorbance microplate reader at OD = 570 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

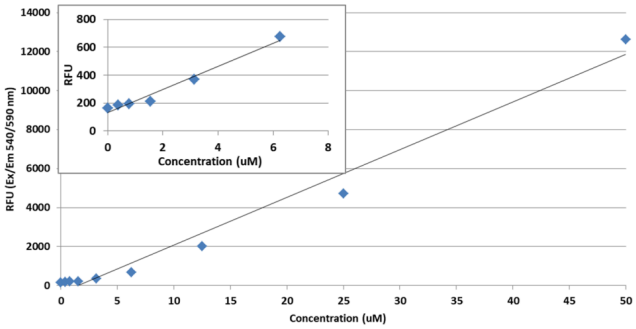

Figure 1. Lactose dose response was measured with the Amplite® Lactose Quantification Ion Kit in a 96-well solid black plate using Fluorimetric analysis. The measurement was performed with SpectraMax (GEMINIXPS from Molecular Devices) fluorescence plate reader.

APPENDIX

Lactose Standard for Colorimetric Analysis

Add 50 µL of a diluted Lactose Standard stock solution (1 mM) to 200 µL of Assay Buffer (Component B) to make a 200 µM (LS1) dilution. Then perform 1:2 serial dilutions to get serially diluted Standard (LS2 – LS7).

DISCLAIMER

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