# Lactose Colorimetric/Fluorometric Assay Kit

(Catalog #K624-100; 100 assays; Store at -20°C)

## I. Introduction:

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 the BioVision Lactose Assay Kit, Lactose is hydrolyzed to glucose and galactose. The galactose is subsequently oxidized generating color (OD 570 nm) 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 (serum, plasma, other body fluids, food, growth media, etc.). Pretreatment of samples is not required. The kit can be used as a high throughput assay.

## II. Kit Contents:

| Components | K624-100 | Cap Code | Part No. |
|---|---|---|---|
| Lactose Assay Buffer | 25 ml | WM | K624-100-1 |
| Probe (DMSO solution) | 0.2 ml | Red | K624-100-2A |
| Lactase (Lyophilized) | I Vial | Blue | K624-100-4 |
| Lactose Enzyme Mix (Lyophilized) | 1 Vial | Green | K624-100-5 |
| HRP (Lyophilized) | 1 Vial | Purple | K624-100-6 |
| Lactose Standard (100 nmol/µl) | 100 µl | Yellow | K624-100-7 |

## 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 100nmol/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/µl by adding 10 µl of the Lactose Standard to 990 µl of Lactose Assay Buffer and mix well. Then take 20 µl into 180 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 volume to 50 µl/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 µl) can be directly added to the wells, then adjust the total volume to 50 µl 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 µl 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 µl of Lactase to one well, add 2 µl 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 µl Reaction Mix containing:

   44 µl Lactose Assay Buffer
   2 µl Probe*
   2 µl Lactose Enzyme Mix
   2 µl HRP

   ***Notes:** The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Using 0.4 µl 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 µl 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 nmol/µl or µmol/ml 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

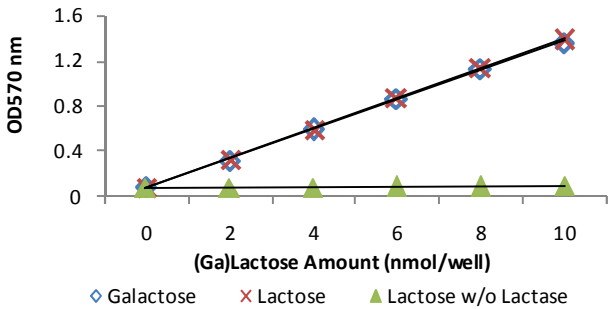


**Figure Legend: Lactose Standard Curve.** Assays were performed following the kit instructions. The kit detected galactose and lactose equally. In the absence of Lactase, the kit detected galactose, but not lactose.

***FOR RESEARCH USE ONLY! Not to be used on humans.***

## GENERAL TROUBLESHOOTING GUIDE:

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Use of ice-cold assay buffer | • Assay buffer must be at room temperature |
| | • Omission of a step in the protocol | • Refer and follow the data sheet precisely |
| | • Plate read at incorrect wavelength | • Check the wavelength in the data sheet and the filter settings of the instrument |
| | • Use of a different 96-well plate | • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates |
| Samples with erratic readings | • Use of an incompatible sample type | • Refer data sheet for details about incompatible samples |
| | • Samples prepared in a different buffer | • Use the assay buffer provided in the kit or refer data sheet for instructions |
| | • Samples were not deproteinized (if indicated in datasheet) | • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated |
| | • Cell/ tissue samples were not completely homogenized | • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope |
| | • Samples used after multiple free-thaw cycles | • Aliquot and freeze samples if needed to use multiple times |
| | • Presence of interfering substance in the sample | • Troubleshoot if needed, deproteinize samples |
| | • Use of old or inappropriately stored samples | • Use fresh samples or store at correct temperatures till use |
| Lower/ Higher readings in Samples and Standards | • Improperly thawed components | • Thaw all components completely and mix gently before use |
| | • Use of expired kit or improperly stored reagents | • Always check the expiry date and store the components appropriately |
| | • Allowing the reagents to sit for extended times on ice | • Always thaw and prepare fresh reaction mix before use |
| | • Incorrect incubation times or temperatures | • Refer datasheet & verify correct incubation times and temperatures |
| | • Incorrect volumes used | • Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | • Use of partially thawed components | • Thaw and resuspend all components before preparing the reaction mix |
| | • Pipetting errors in the standard | • Avoid pipetting small volumes |
| | • Pipetting errors in the reaction mix | • Prepare a master reaction mix whenever possible |
| | • Air bubbles formed in well | • Pipette gently against the wall of the tubes |
| | • Standard stock is at an incorrect concentration | • Always refer the dilutions in the data sheet |
| | • Calculation errors | • Recheck calculations after referring the data sheet |
| | • Substituting reagents from older kits/ lots | • Use fresh components from the same kit |
| Unanticipated results | • Measured at incorrect wavelength | • Check the equipment and the filter setting |
| | • Samples contain interfering substances | • Troubleshoot if it interferes with the kit |
| | • Use of incompatible sample type | • Refer data sheet to check if sample is compatible with the kit or optimization is needed |
| | • Sample readings above/below the linear range | • 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. | | |