# Lactose Assay Kit

**Catalog No.: abx298958**

**Size:** 96 tests

**Storage:** Store Assay Buffer 1, Assay Buffer 2, Reaction Buffer, and Standard at 4°C. Store Dye Reagent at 4°C and keep in dark. Store Enzyme 1 and Enzyme 2 at -20 °C.

**Application:** For quantitative detection of Lactose concentrations in serum, plasma, cell lysates, cell culture supernatants, urine, saliva, milk, and other biological fluids.

**Detection Range:** 0.2 mmol/L – 20 mmol/L

**Introduction:** Lactose is a disaccharide composed of glucose and galactose which are joined by a glycosidic linkage. Abbexa's Lactose Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Lactose concentrations. Lactose is hydrolyzed by lactase (β-galactosidase), releasing galactose and glucose. Glucose is hydrolyzed by glucose oxidase, releasing $H_2O_2$ which reacts with the kit's dye reagent to create an absorption maximum at 505 nm. The intensity of the color is proportional to the concentration of Lactose, which can then be calculated.

**Kit components**

1. 96 well microplate
2. Assay Buffer 1: 30 ml
3. Assay Buffer 2: 30 ml
4. Enzyme 1: 1 vial
5. Enzyme 2: 2 vials
6. Reaction Buffer: 15 ml
7. Dye Reagent: 2 vials
8. Standard: 1 vial
9. Plate Sealer: 3

**Materials Required But Not Provided**

1. Microplate reader (505 nm)
2. Incubator or Convection Oven
3. Distilled Water
4. Pipettor, multi-channel pipettor
5. Pipette tips
6. Mortar
7. Centrifuge
8. Timer

## Protocol

### A. Preparation of Sample and Reagents

**1. Reagents**

- **Dye Reagent Solution**
  Add 10 ml of distilled water into the Dye Reagent vials and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.
- **Standard Solution**
  Add 1 ml of distilled water into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 500 µl of this solution and add 500 µl of distilled water to prepare the Standard Solution (concentration 20 mmol/L). Unused Standard Solution can be stored at 4°C.
- **Enzyme 1 Solution**
  Add 1 ml of Reaction Buffer into the Enzyme 1 vial and mix thoroughly to prepare the Enzyme 1 Solution. Ensure that the Enzyme 1 has completely dissolved prior to use.
- **Enzyme 2 Solution**
  Add 1 ml of Reaction Buffer into the Enzyme 2 vial and mix thoroughly to prepare the Enzyme 2 Solution. Ensure that the Enzyme 2 has completely dissolved prior to use.

**2. Sample**

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 500 µl distilled water for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Then add 250 µl of Assay Buffer 1 and 250 µl of Assay Buffer 2 and mix again. Centrifuge at 10,000 rpm for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 0.5 ml of distilled water and transfer it into the centrifuge tube. Then add 250 µl Assay Buffer 1 and mix, followed by 250 µl Assay Buffer 2 and mix again. Centrifuge at 10,000 rpm for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

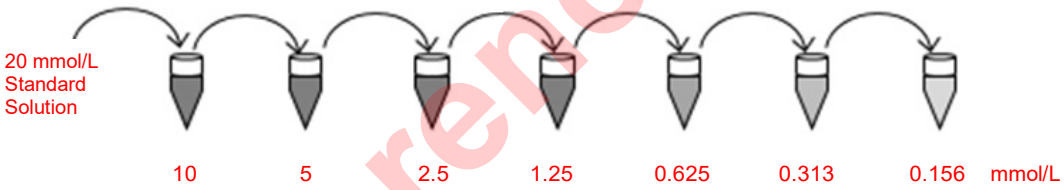
- **Liquid samples**
  Serum and Plasma samples can be assayed directly. Milk samples should be prepared by mixing 500 µl of sample with 250 µl Assay Buffer 1 and 250 µl Assay Buffer 2. Centrifuge at 10,000 rpm for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately. Note that the dilution factor is 2 for samples prepared in this way.

**B. Assay Procedure**

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 7 tubes with 10 mmol/L, 5 mmol/L, 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.313 mmol/L, and 0.156 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 20 mmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.




2. Set the sample, standard, control and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard, sample and control in duplicate. Each sample requires a control.
3. Add 60 µl of Reaction Buffer to all wells.
4. Add 20 µl of sample to the sample and control wells.
5. Add 20 µl of prepared standards to the standard wells.
6. Add 20 µl to distilled water to the blank wells.
7. Add 10 µl of Enzyme 1 Solution to the sample wells, standard wells and blank wells.
8. Add 10 µl of distilled water to the control wells.
9. Tap the plate gently to mix, then cover the plate with a plate sealer. Incubate at 37 °C for 30 minutes.
10. Add 10 µl of Enzyme 2 Solution to all wells.
11. Add 100 µl of Dye Reagent Solution to all wells.
12. Tap the plate gently to mix, then cover the plate with a plate sealer. Incubate at 37 °C for 20 minutes.
13. Read and record absorbance at 505 nm.

**C. Calculations**

Lactose concentration per mg of protein:

$$\mathbf{Lactose\ (\mu mol/mg)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{20}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Lactose concentration per g of sample:

$$\mathbf{Lactose\ (\mu mol/g)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{20}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Lactose concentration per $10^4$ cells or bacteria:

$$\textbf{Lactose}\ (\boldsymbol{\mu mol/10^4\ cells}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{N}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{20}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Lactose concentration per ml serum or plasma:

$$\textbf{Lactose}\ (\boldsymbol{\mu mol/ml}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \mathbf{20} \times \boldsymbol{n} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

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|---|---|
| $\mathbf{C_{Protein}}$ | Concentration of protein (in mg/ml) |
| $\mathbf{C_{Standard}}$ | Concentration of highest standard (20 mmol/L = 20 µmol/ml) |
| $\mathbf{W}$ | Weight of the sample (in g) |
| $\mathbf{N}$ | Number of cells or bacteria (× $10^4$) |
| $\mathbf{V_{Assay}}$ | Volume of distilled water, Assay Buffer 1 and Assay Buffer 2 used in sample preparation (1 ml) |
| $\mathbf{V_{Sample}}$ | Volume of sample (0.02 ml) |
| $\mathbf{V_{Standard}}$ | Volume of standard (0.02 ml) |
| $\boldsymbol{n}$ | Dilution factor |

### D. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.