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# **Lactose Quantification with Fluorometer 530/590nm**

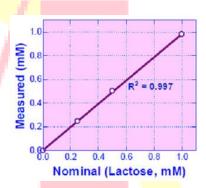
#### **Description:**

LACTOSE (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>), also called milk sugar, is a disaccharide that consists of  $\beta$ -D-galactose and  $\alpha/\beta$ -D-glucose through a  $\beta$ 1-4 glycosidic linkage. Lactose is the major sugar and makes up 2–8% of milk. Simple, direct and high-throughput assays for lactose determination find wide applications. Assay uses specific enzyme-coupled reactions in which lactose is cleaved and the resulting galactose forms a colored product. The fluorescence intensity at 590nm when excited by 530nm is directly proportional to the lactose concentration in the sample.

## **Assay Performance:**

- Linear Detection Range: 0.04 to 1 mM.
- Detection Limit: 0.04 mM (1.4 mg/dL, 14 ppm).
- Typical Precision (CV%): <5%.





### **Assay Procedure:**

Important: prior to assay, bring the assay reagents to room temperature. Add 120 μL dH<sub>2</sub>O to Enzyme Mix and 120 μL dH<sub>2</sub>O to Lactase tubes. Mix well by pipetting and vortexing. Keep enzyme tubes cold during the assay.

- 1. Prepare 1 mM Lactose Standard by mix 10 μL provided Standard with 190 μL H<sub>2</sub>O. In separate mini-glass tubes, add 10 μL H<sub>2</sub>O ("Blank"), 10 μL 1 mM Standard ("Std"), and 10 μL Sample.
- 2. Prepare enough Working Reagent by combining the following per tube: 90 μL Assay Buffer, 1 μL Enzyme Mix 1 μL Lactase and 1 μL Dye Reagent. Mix well. Add 90 μL Working Reagent to each tube. Incubate for 30 min at room temperature in the dark.
- 3. Switch on the fluorometer. To calibrate the fluorometer, place the "Blank" tube into the sample holder. Press "Calibrate", "Assay 1", then "Blank". Fluorometer starts Measuring. Press number setting keys in "< + >" until the window shows "1.00". Place the "Std" tube into the Sample holder. Press "Measure". The fluorometer shows "Calibrate Finished". The Fluorometer is now calibrated. Press "Return".
- 4. Measure. Place the sample tube into the Sample Holder. Press "Measure" → "Assay 1" → "Measure". The Lactose concentration will be displayed in the window. Record the data, or press "Save" to save the data for later retrieval. Press "Return" and then "Measure" for the next sample.

Note: if Sample concentration is higher than the upper limit, dilute Sample in H<sub>2</sub>O and repeat assay.

#### **Product Information:**

- Lactose Assay Kit: Sufficient for approximately 100 assays. Kit content: (1) 10 mL Assay Buffer, (2) Enzyme Mix (dried), (3) Lactase (dried),
  (4) 120 μL Dye Reagent, (5) 1 mL Standard, (6) 100 mini glass tubes.
- The Fluorometer comes with a 5VDC power adapter, a USB cable, manual and data management software CD.
- Mini glass tubes(optional).
- \*Avoid contact and inhalation. Standard laboratory safety procedures should be followed when handling this product. Safety procedures include wearing OSHA approved safety glasses, gloves and protective clothing.
- \*Shipping and storage: the kit is shipped on ice. Store Assay Buffer at 4°C and other reagents at -20°C. Shelf life of 12 months after receipt.