Industrial Lactose Analysis

A simple two-step enzymatic assay for lactose in aqueous solutions or extracts.

Bulletin Reference	TB – USA-Lactose – Industrial – GMRD-095 – V.02
Order Code	GMRD-095 (For GM8, GL6)
Reagent Kit Size	80 ml (120 analyzer cycles) – GMRD-095
Instruments	All GM8 and GM10 series analyzers N.B.: Lactose analysis may also be performed via the glucose assay on GL6 analyzers.
Samples	Aqueous solutions and extracts
Sample Volume	10 µl
Analysis Time	20 seconds (from injection)
Working Range	0.5 - 20 %W/V (GM8, GL6)
Reagent Stability	Shelf-life unopened: 9 months stored at 0 - 5°C. Aliquots may be frozen for extended life.
Note	Sample opacity or turbidity presents no problem since the detection method is electrochemical rather than spectrophotometric. Endogenous glucose, if present, should be determined as a sample 'blank', i.e. extract diluted pro-rata in water instead of ß-galactosidase. Incomplete hydrolysis may take place for lactose concentrations greater than 10 %W/V. For greater accuracy at these levels repeat hydrolysis using a 5 µl sample and scale results proportionally.

Principle

i) Lactose (milk sugar), a disaccharide, is stoichiometrically hydrolysed by β-galactosidase (β-GAL) to α-D-glucose and galactose in a simple pre-reaction. Under the special buffer conditions used, mutarotation to β-D-glucose rapidly occurs,

$$\alpha\text{-Lactose} \xrightarrow{\beta\text{-Galactosidase } (\beta\text{-GAL})} (\alpha\text{-D-Glucose}) \xrightarrow{\text{Phosphate Buffer}} \beta\text{-D-Glucose} + \text{Galactose}$$

ii) In the presence of molecular oxygen, β-D-glucose is oxidised by the enzyme glucose oxidase (GOD) to gluconic acid and hydrogen peroxide,

$$\beta\text{-D-Glucose} + O_2 \xrightarrow{\text{Glucose Oxidase (GOD)}} \text{D-Gluconic acid} + H_2O_2$$

Under the conditions of the assay, the rate of oxygen consumption is directly proportional to glucose concentration, which relates directly to the original lactose concentration.