CUSTOMBIOTECH

Roche

Lactose Assay for Cedex® Bio & Bio HT Analyzers

Reliable and convenient determination in process control

The Cedex® lactose assay is designed for automated testing on Cedex® Bio and Cedex® Bio HT Analyzers, providing fast and precise determination in various aqueous samples.

Applications

1. Cell culture and microbial fermentation

When lactose serves as a nutrient in culturing processes, the Cedex® Lactose assay allows for accurate monitoring of the concentration. This is vital for implementing sophisticated feeding strategies, preventing nutrient depletion or overflow.

2. Food industry

In food manufacturing, especially dairy production, fast and accurate lactose measurement is required for process and quality control. The Cedex® Lactose assay provides a reliable and convenient solution for:

- Determining lactose content in dairy products, convenience food and other processed goods.
- Optimizing the production of “lactose-free” products, ensuring regulatory compliance, and minimizing costs and time associated with the enzymatic hydrolysis of lactose.

Ideal for process control

- Fully automated assay
- Ready-to-use reagents
- No sample pretreatment required for pipettable aqueous solutions
- Ability to analyze more parameters on the same platform

Wide concentration range for various applications

In order to provide highly accurate results over a wide lactose concentration range of 0.14 g/L up to the maximal solubility, there are two instrument protocols, for a low range providing high sensitivity, and a high range for higher concentrated samples.

Protocol	Lactose concentration range
LCOB	0.137 - 20.5 g/L, 0.4 - 60 mmol/L
LCOD	1.37 - 171 g/L, 4 - 500 mmol/L

Assay principle

Lactose is hydrolyzed into glucose and galactose by β-galactosidase. Hexokinase (HK) then phosphorylates glucose using ATP to glucose-6-phosphate (G-6-P), which is oxidized by glucose-6-phosphate dehydrogenase (G6P-DH) with reduction of $NADP^+$ to NADPH. The rate of NADPH formation measured by the UV photometer is directly proportional to the lactose concentration of the sample.

$$\text{Lactose} + H_2O \xrightarrow{\beta\text{-galactosidase}} \text{D-Glucose} + \text{D-Galactose}$$

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + NADP^+ \xrightarrow{\text{G6P-DH}} \text{D-Gluconate-6-phophate} + \text{NADPH} + H^+$$

For use in quality control only.

Correction of glucose interference

Free glucose in samples will contribute to the signal in the lactose assay, according to the reaction scheme above. If relevant glucose concentrations are anticipated in your samples, we advise correcting the lactose result by subtracting the additionally determined glucose concentration (concentrations in mmol/L !). The glucose interference can be automatically compensated using a ratio setting in the Cedex® software. Refer to the lactate kit instructions for details.

Cross-reactivities

Except for lactose and glucose, other carbohydrates do not interfere with the assay. The following substances were tested at concentrations up to 100 mmol/L and showed no impact on the lactose assay result:

- Sucrose
- Galactose
- Fructose
- Mannose
- Trehalose
- Glucosamin

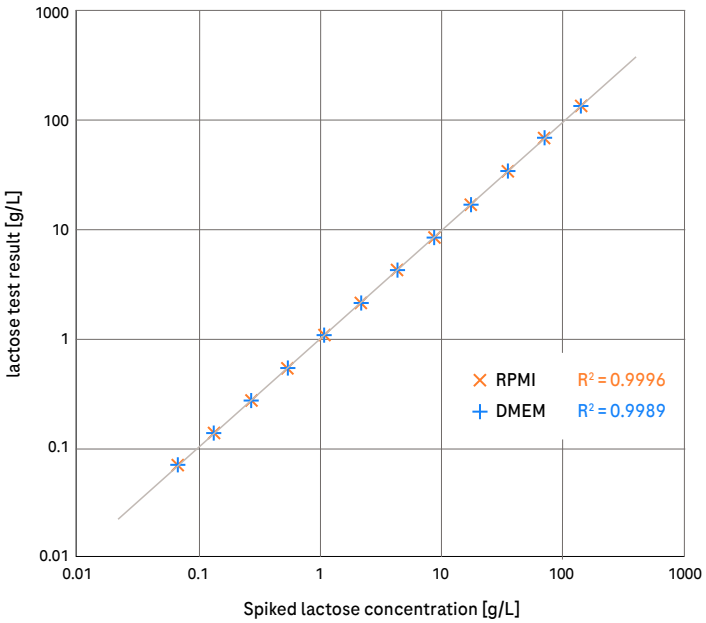
Accurate and precise measurement

The Cedex® assay accurately determines lactose concentrations across a wide range, as demonstrated by the perfect linearity and recovery in spiked cell culture media (see figure 01).

Representative precision data were obtained on a Cedex® Bio HT Analyzer with samples of three concentration levels. Coefficients of variation (CV) were determined for in-run precision (n=21) and inter-run precision (on 10 days):

	Level 1	Level 2	Level 3
Mean	0.7 g/L	8 g/L	41 g/L
CV in-run	0.8 %	0.4 %	0.9 %
CV inter-run	1.7 %	1.4 %	1.8 %

(Verification data of Roche Diagnostics)



01

Accuracy. Two types of cell culture media were spiked with a row of lactose concentrations and determined on a Cedex® Bio Analyzer. Over the whole range, the results show a perfect linearity and a recovery within ± 5 % of the target values. (Verification data of Roche Diagnostics)

Food testing applications

For determination of the lactose content in various food materials, the sample has to be applied as an aqueous solution which can be transferred by the pipetting needle of the Cedex® Analyzer.

Direct testing:

Suspensions (e.g., cell cultures, microbial fermentation) and emulsions (e.g., milk) can be tested without any sample preparation, if particles are very small and nonaqueous content does not separate from the aqueous phase. Only 2 µL of a sample are used in the assay reaction volume of 201 µL, making the assay robust against colored or potentially inhibitory ingredients.

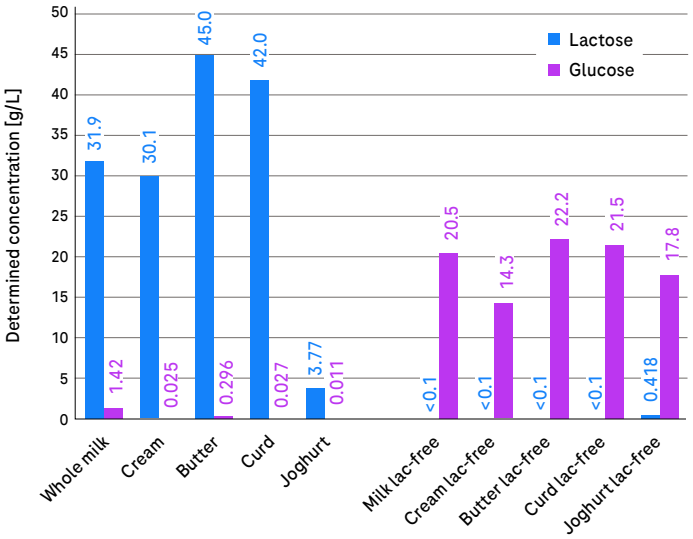
Samples requiring prior preparation:

Oily samples or samples containing larger particles or organic solvents must not be applied directly to a Cedex® Analyzer. Such types of samples require a prior extraction with water and separation from the nonaqueous components. For foodstuffs like butter, cheese, sausages, chocolate, or ice cream, established extraction procedures are described in the technical literature.

The common lactose threshold for food designated as "lactose-free" is ≤1 g/L or ≤1 g/kg. The Cedex® lactose assay has a lower limit of 0.137 g/L, which is sensitive enough for testing extracts of complex sample types.

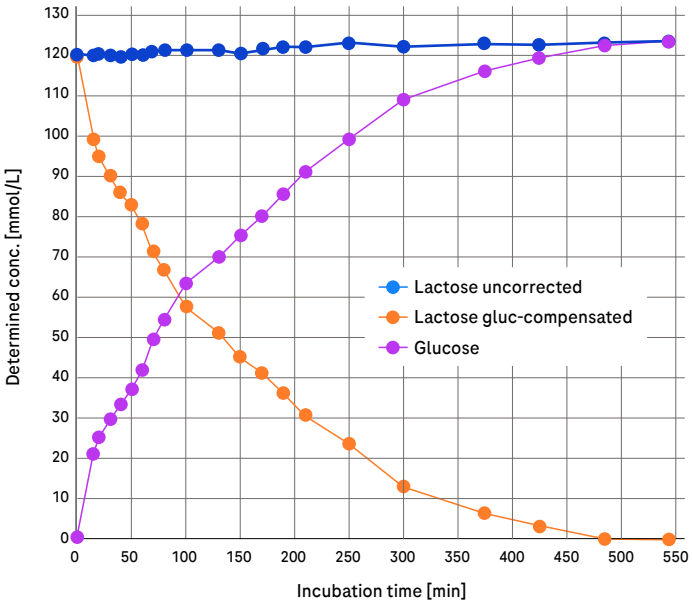
Glucose correction:

After lactose digestion the resulting products often contain a high concentration of glucose. Then the lactose result (in mmol/L) needs to be corrected by subtracting the glucose concentration. The Cedex® software can be preset for parallel determination of lactose and glucose and automated calculation of the corrected lactose result value.



02

Lactose/Glucose in dairy products. Lactose and glucose were determined using a Cedex® Bio Analyzer in untreated and "lactose-free" dairy items from a supermarket. Butter and curd were extracted with water and after centrifugation the aqueous phase was analyzed. The lactose results were adjusted by subtracting the glucose background. (Verification data of Roche Diagnostics)



03

Process control of lactase treatment. Lactase enzyme was added to whole milk. During the incubation, lactose and glucose were determined at short intervals using a Cedex® Bio HT Analyzer. The lactose results were adjusted by subtracting glucose. The results clearly show the progression of the lactose digestion. By monitoring the decrease in lactose, such a treatment can be optimized regarding the required enzyme amount, incubation temperature, and time to achieve the targeted depletion. (Verification data of Roche Diagnostics)

Ordering information
For determination of lactose the following products are required in addition to the Cedex® instrument with the general system reagents and accessories:

Product	Pack size	Catalog Number
Lactose Bio	4 x 100 tests	10 387 653 001
Lactose Bio HT	400 tests	10 387 661 001
Calibrator D Bio	6 x 1 mL	07 368 321 001
Control D Level 1 Bio	6 x 1 mL	07 368 178 001
Control D Level 2 Bio	6 x 1 mL	07 368 186 001
Control D Level 3 Bio	6 x 1 mL	07 368 194 001

Optional for correction of glucose background

Product	Pack size	Catalog Number
Glucose Bio	2 x 100 tests	06 343 732 001
Glucose Bio HT	800 tests	06 608 418 001
Calibrator A Bio	6 x 1 mL	06 682 189 001
Control A Level 1 Bio	6 x 1 mL	06 682 197 001
Control A Level 2 Bio	6 x 1 mL	06 682 227 001
Control A Level 3 Bio	6 x 1 mL	06 682 545 001

Regulatory Disclaimers
are listed on the respective product page on custombiotech.roche.com

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Scan for ordering information for all Cedex® Bio Analyzer and Cedex® Bio HT Analyzer assays

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