lactosens®

Biosensor for the detection of residual lactose in lactose reduced and lactose free milk

Product Relaunch Documentation

directsens Biosensors

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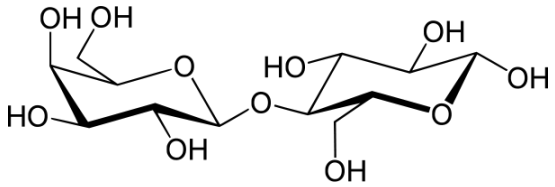
Background Information

Lactose

Lactose is a sugar which is naturally present in milk products. The disaccharide derived from galactose and glucose, makes up around 2–8% of mammalian milk[1], with the amount varying between species and individuals[2]. The name comes from *lact*, the Latin word for milk, plus the ending *-ose*, used to name sugars.



Lactose: IUPAC name:
β-D-galactopyranosyl-(1 4)-D-glucose
CAS Number: 63-42-3
Chemical formula: $C_{12}H_{22}O_{11}$
Molar mass: 342.30 g/mol

Lactose intolerance[3,4]

During the first year of human life lactose is the most important source of energy. In the digestive system the enzyme lactase catalyzes the hydrolysis of lactose into glucose and galactose which can be easily absorbed. In the course of life many humans lose their ability to digest lactose or this ability is reduced due to a lower production of lactase. Only 25% of world`s human population are lactose persistent, meaning that the lactase production remains high during adulthood. The loss of lactase activity leads to the medical condition called lactose malabsorption or lactose intolerance[5]. In most cases, the deficiency causes symptoms which may include abdominal bloating and cramps, flatulence, diarrhea, nausea, etc. after consuming significant amounts of lactose[6].

Most people with lactose intolerance can tolerate some amount of lactose in their diet and do not need to avoid milk or milk products completely. Individuals vary in the amount of lactose they can tolerate. Research suggests that adults and adolescents with lactose malabsorption could eat or drink at least 12 grams of lactose in one sitting without symptoms or with only minor symptoms. This amount is the amount of lactose in 1 cup of milk[7].

Lactose-free milk and its production

In the recent years an increasing demand for "free from" food specialties can be recognized. Consumers tend to expect beneficial effects for their health, avoiding certain food components. One of these components is lactose. Currently, the market for lactose-free dairy products is growing approximately 20 percent annually[8]. A large number of lactose-free dairy products are available for people suffering from lactose intolerance or for people who expect a beneficial health effect. In the milk processing industry lactose is mainly removed by means of enzymatic hydrolysis with the enzyme lactase. The enzyme splits the disaccharide lactose in its compounds galactose and glucose, which are types of sugar that can be easily utilized by the human digestive system.

The production process of lactose-free milk[9],[10],[11] can be performed in two ways:

Batch production in tanks

The most common method is the addition of soluble lactase to pasteurized milk in tanks. This mixture is stirred in a temperature-controlled tank and the reaction takes place for a certain time until the lactose concentration is below the desired threshold level. This process is influenced by various factors (temperature, pH, initial material), resulting in slightly varying production times.

Inline dosing production

This method to produce lactose free milk is only suitable for long-life (UHT) milk. Lactase is dosed inline aseptically to UHT milk and the process of lactose cleavage is than performed in the milk packages. This process lasts for approximately 5 days under standardized conditions in the dairy company. The milk/lactase ratio has to be optimized so that the lactose concentration after this time period is below the threshold. The method reduces

1 American Dairy Products Institute 2002
2 National Dairy Council 1993
3 National Institute of Diabetes and Digestive and Kidney Diseases 2014
4 Vesa et al. 2000
5 Ingram, Catherine J. E. et al. 2009
6 Järvelä et al. 2009
7 Suchy et al. 2010
8 Global Lactose-free Food market 2016-2020 2016
9 Jelen und Tossavainen 2003
10 Harju et al. 2012
11 Fiechter et al. 1980

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the amount of lactase needed and adding the lactase after heat treatment avoids undesired Maillard reactions (browning of the milk).

Limits

The threshold value for the residual lactose concentration is not standardized in an EU-wide regulation, although the European Food Safety Authority (EFSA) already did some research on thresholds leading to symptoms in lactase deficient patients[12].

So far, only national and regional regulations or recommendations exist. Due to the lack of clear rules on the use of claims to indicate the absence or reduction of lactose in foods, manufacturers across Europe can set their own management thresholds for labelling foods suitable for people with lactose intolerance. Depending on the country lactose concentrations of < 1 g/L or < 0.1 g/L are required in order to be permitted to label the product "lactose-free".

Lactase

Lactase is an enzyme produced by many organisms. In humans and other mammals, it is located in the brush border of the small intestine[13]. Lactase is essential for the complete digestion of whole milk, as it breaks down lactose. In the dairy industry, lactase can be purchased as a food supplement, and is added to milk to produce "lactose-free" milk products[14]. Lactase for industrial usage[15] originates mostly from microorganisms due to their higher performance and lower price compared to lactases from other sources. At the moment most producers of lactose-free milk are using yeast neutral lactases from *Kluyveromyces lactis,* which are sold under various brand names (HA lactase, Maxilact, opti-lactase LX 2 or Lactozym Pure). Beside their ability to cleave the disaccharide lactose into glucose and galactose the lactases originating from yeasts can also catalyze a side reaction called transgalactosylation. During this process galactose is added to lactose forming so called galactosyl-oligosaccharides (GOS).

Currently a new class of lactases is launched by a range of enzyme suppliers. Originating from *Bifidobacterium bifidum* this new enzyme differs considerably from traditional yeast lactases. The enzyme also works at lower pH and the GOS formation is dramatically reduced.

GOS

During the production of lactose-free milk, residual transgalactosylation activities of yeast neutral lactases lead to the formation of small amounts of GOS[16]. In contrast to other methods for lactose determination (e.g. enzymatic methods) where the GOS formation results in an extensive overestimation of the lactose concentration DirectSens® has chosen a novel approach. The already small influence of the GOS formation on the lactose determination with LactoSens® is further minimized by a calibration function based on extensive studies, comparing results of HPLC determinations to LactoSens® results. The available validation report proofs that this approach is valid for the above mentioned yeast neutral lactases.

Lactose Determination

The challenge for the dairies in process and quality control is to detect remaining lactose concentrations at the resulting low levels. An exact detection of the remaining lactose concentration is important to optimize production time and costs, and is an important parameter for quality control. Determination of lactose in milk goes way back. Already in 1918, a colorimetric and a titration method were described[17].

Since then several methods based on different measurement principals have been established for routine analysis of lactose in whole milk[18],[19],[20],[21]. But when it comes to the detection of traces of residual lactose in lactose-free milk products most of these methods reach their limits. Due to the lack of accurate alternatives, dairy companies are currently using mainly three methods. Enzymatic methods, which are based on the photometric detection of glucose or galactose, need laborious sample preparation including several pipetting and incubation steps and are prone to interference by GOS. Cryoscopy, a method relying on changes in the freezing point due

12 EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2010
13 U.S. National Library of Medicine 2016
14 Woychik und Holsinger 1977
15 Neelakatnan et al. 1999
16 Mahoney 1998
17 Folin und Denis 1918
18 Essig und Kleyn 1983
19 Kleyn 1985
20 Kouaouci 2007
21 Perati et al. 2015

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to different lactose concentrations, is very matrix dependent and inaccurate. Currently HPLC analysis can be seen as the gold standard for lactose determination. Since HPLC analysis is mainly performed by external analytical laboratories, the analysis is expensive and the time-to-result is long.

Due to the lack of a fast, precise and easy method, DirectSens® has developed the LactoSens® Biosensor Test Kit for the detection of residual lactose in lactose-free and lactose-reduced milk. Biosensors are highly specific measuring devices, which use enzymes to specifically detect a substance in a sample and convert it into a measurable electric signal.

LactoSens Biosensor Test Kit

DirectSens® developed a biosensor for the detection of residual lactose in lactose-free milk. The core element of the sensor is an optimized enzyme, which is immobilized on a disposable test strip. Using the LactoSens® Reader and the LactoSens® Software, the lactose concentration can be measured directly and accurately. The system convinces by its simple handling and sample preparation. *The lactose results are obtained in less than a minute.*

The sample is prepared by a simple 1:5 dilution with the ready-to-use dilution buffer, which is included in the kit. 100 µL of the diluted sample are placed on one of the single use test strip carrying the immobilized enzyme. During the measurement, lactose in the sample is oxidized to lactobionic acid and the transferred electrons are measured directly by a potentistatic reading device called LactoSens® Reader.

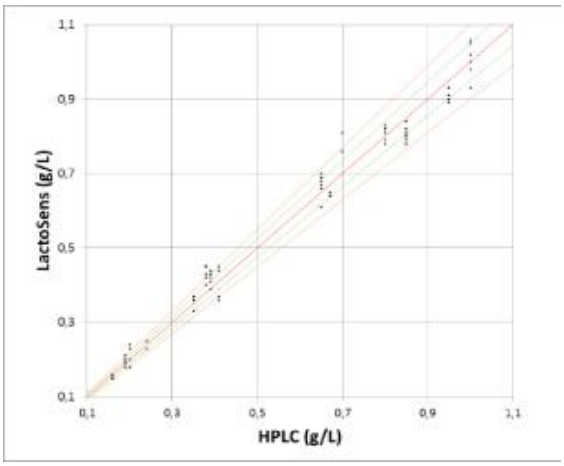
Every sensor batch has its own calibration curve, which will be used by the software for the quantification of lactose in milk samples. The calibration data are transferred to the reader software by means of QR code. The method is calibrated for the range of 0.008 % to 1% lactose. The correctness of the yielded results was validated against HPLC analysis.

To verify the correctness of the quantitative results, various HPLC-analyzed milk samples were measured over the quantitation range. The samples were taken from lactose hydrolysis reactions employing different lactases, temperatures and milk fat contents.

The method is fully validated for milk, with application notes for other white dairy products under development.

DirectSens®

DirectSens® is a company dedicated to the development of 3rd generation biosensors. Founded to convert scientific results in innovative solutions for the detection of sugars, it has now successfully introduced the first product to the market. Over the last few years DirectSens® has developed a technology platform that can be used for the detection of various carbohydrates. The flexibility of the technology along with the simple architecture enables DirectSens® to target several markets where innovation in sensor technology is requested. DirectSens® was founded by a team of skilled scientists and as been completed by experienced product developers and dedicated executives. An international advisory board constantly challenges the team and reviews the progress.



Comparing the results of lactose determinations using HPLC versus the LactoSens® biosensor

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The LactoSens® Biosensor System Manual

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LactoSens®Test Kit Specifications

- Single use biosensors
- Product number LK0125 (25 biosensors) / LK0110 (10 biosensors)
- Sample preparation: 1:5 dilution with buffer
- Sample volume: 100 µL
  Measuring time: < 1 minute
- Quantitation range: 0.008% - 1% lactose in milk
- Limit of Quantitation: 0.008 % lactose equivalent to 0.08 g/L
- Storage: 2-8°C

Content of LactoSens®Test Kit

- 25/10 single packed LactoSens® Biosensors
- 2/1 bottles (125 mL each) of ready-to-use LactoSens® Buffer (LB01)
- 1 vial (2 mL) of positive control (lactose solution), ready to use
- Manual
- Quality Assurance Certificate

Required but not included in the LactoSens®Test Kit

- LactoSens® Reader (LR01) with LactoSens® Adapter (LA01)
- LactoSens® Software
- Vials, pipettes and tips for sample dilution
- 100 µL pipette and pipette tips for sample application
- Vortex devices for mixing samples
- Laptop or PC for connection with LactoSens® Reader

Precautions

Use the LactoSens® system at room temperature (22 ± 2°C).
Avoid spilling liquids into the Reader. This may cause defects or destruction of the instrument.

Installation of LactoSens® Software

Install LactoSens® software on your PC or laptop according to the LactoSens® Software Installation Guide.

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Determination of lactose in lactose reduced and lactose free milk with LactoSens®

1. General preparations

- Take a representative sample of milk.
- Bring sample, buffer, positive control and necessary number of sensors to room temperature (22 ± 2°C).
- Insert the LactoSens® Adapter into the LactoSens® Reader.
- Connect the LactoSens® Reader and the USB camera to the PC with the cables supplied.
- Switch on the LactoSens® Reader by pressing the start button for approx. 2 seconds until the white ring around the start button is illuminated.

2. Sample preparation

- Mix milk sample (e.g. by vortexing).
- Dilute milk sample 1:5 with LactoSens® Buffer (e.g. 1 mL sample plus 4 mL buffer).
- Mix diluted sample (e.g. by vortexing).

3. Measurement

The LactoSens® Software will guide you through the whole measurement process with step-by-step instructions.

- Start the LactoSens® Software on your computer.
- A green icon in the lower left corner of the LactoSens® Software window will indicate a connected LactoSens® Reader. If no connection can be found, the software will prompt you to connect/switch on the reader.
- Enter the name or ID of the sample to be measured (if no name is entered, the Software will use a default name) and press the button "Scan Sensor QR Code".
- Select the provided USB-camera from the dropdown list and scan the QR code on the package of the sensor (detailed instruction for the scanning process is given in Section 6 QR code). This scan will enter the lot specific calibration data, needed for measurement and the expiry date of the sensor.
- If scanning is not possible, the necessary calibration data (the 53-digit number without spaces printed below the QR code on the package of the sensor) can be entered manually by clicking the respective box on the screen.
- Once sample name and calibration data are entered, the LactoSens® Software will prompt you to insert the sensor into the reader.
- Unpack the sensor by ripping the silver-foil at the designated spot. Open and take out the sensor.

ATTENTION: DO NOT touch the black area on the sensor!

- Insert the sensor into the adapter by pushing the side with the silver contacts into the adapter slot. The LactoSens® logo has to face upwards.
- Confirm by pressing OK.
- A new window will appear, asking for application of the sample.

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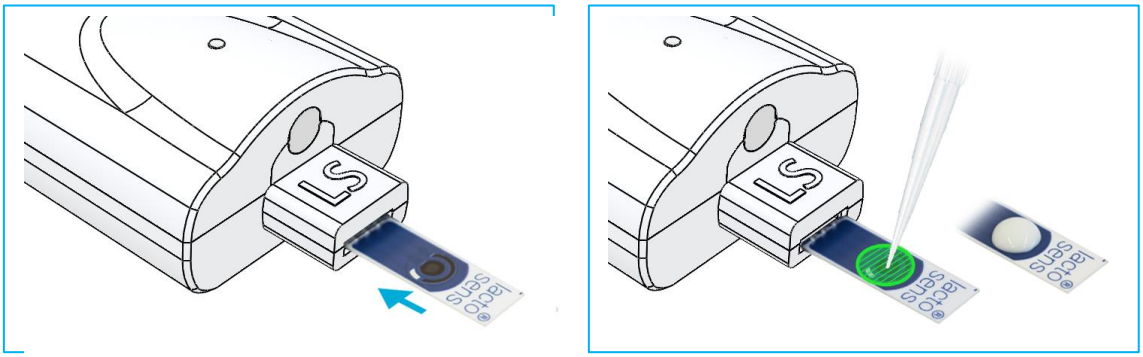
- Pipette 100 µL of the diluted milk sample on the black sensitive area of the sensor. Distribute the sample, as shown in the image below.

ATTENTION: A correct sample application is crucial for an accurate lactose measurement!



Cover black sensor area (here indicated in green)

- After sample application on the sensor press START immediately.

ATTENTION: The time between first contact of the sample with the electrode and pressing START should not exceed 20 seconds!

- A new window with a progress bar will appear and indicate the time to result (approx. 50 seconds).
- After finishing the measurement, the measured lactose concentration will be displayed on the screen. You can switch between g/L or % as recorded units.
- Wipe the milk sample off the sensor with a paper tissue, and remove the sensor carefully from the adapter and discard it. This helps to avoid any accidental spilling of the sample liquid into the adapter.
- Continue by selecting SAVE & NEXT to save the lactose concentration or DISCARD to continue working without saving the result. Both buttons will take you back to the start screen.

  Results will be saved in a comma separated value file (.CSV file) with date, time, sample name, sensor ID, expiry date and measured concentration with unit. The file is located at the default path *C:\Users\[USERS]\Documents\lactosens.csv*. If there is an existing *lactosens.csv* file, the software will automatically amend the values to the file. If no file called lactosens.csv exists, it will be created automatically.

NOTE: The sensors are not reusable!

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4. Using a Positive Control

In order to verify correct handling, the positive control (POC), included in the LactoSens® Test, can be used. The positive control is ready-to-use and can be applied directly to the sensor without any further dilution. The positive control should give you a result of 0.1% ± 0.01% lactose. The use of the positive control is optional.

5. Data storage

All measured results can be stored in a .CSV (comma-separated value) file, which will be automatically generated by the software.

Individual results of the ongoing measurement session can be viewed by clicking on the button "View last results" in the upper left corner. This will open a window with the measurement specific data which can be easily exported to an excel file.

6. QR code

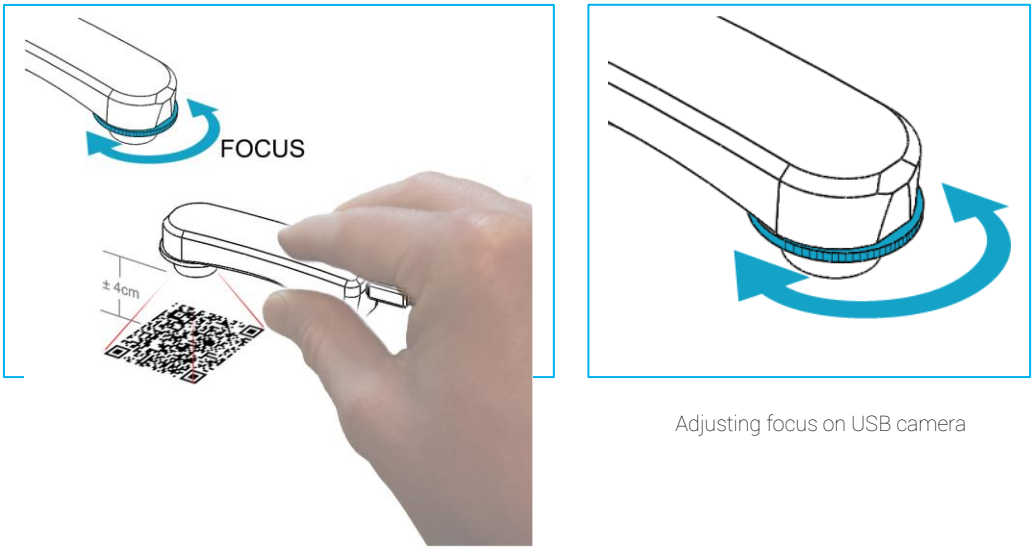
Each individually packed sensor is labeled with a QR code containing the following information:

- Batch specific calibration data
- Sensor expiry date
- Sensor batch name
- Sensor identification number

Before you can start the actual lactose measurement this information has to be transferred to the software. This can either be done by scanning the QR code with the included USB camera or by typing in the 53-digit number (without spaces) printed below the QR code. We strongly recommend to use the USB camera!

6.1. Scanning the QR code

After pressing the button "Scan sensor QR code" the LactoSens® Software will allow you to select the supplied USB camera from the dropdown menu. Once the camera is selected you will see a live view in the software window. Put the package with the QR code in front of you on the table. Take the camera in your right hand as shown in the picture. The sticker on the USB camera shows, how to move the camera and will help you to center the camera above the QR code. Place the camera approximately 1 cm above the QR code and start to move it up slowly. Once the QR code is recognized you will hear a beep and the software will guide you through the next steps.



Adjusting focus on USB camera

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The right focus adjustment of the camera will improve the scanning process. To adjust the focus, place the camera above the QR code at a distance where the QR code matches the red square in the software window. Now adjust the focus by turning the big wheel as shown in the picture above. Once the focus is readjusted use the above mentioned procedure to scan the QR code.

6.2. Enter sensor information manually

After pressing the button "Scan sensor QR code" you can click the box "Enter QR code value manually". Please type in the 53-digit code which is printed below the QR code. Please enter all digits including "." and "-". Do not press space between the digits.

Once the code is recognized you will hear a beep and the software will guide you through the next steps.

6.3 Sensor identification number

Please be aware that the sensors are for single use only. Consecutive measurements with the same sensor are not allowed and lead to strong underestimation of lactose concentration. The unique sensor identification number will help prevent that the sensor is used multiple times and therefore avoid wrong lactose readings.

After pressing the START button in the LactoSens® Software the sensor identification number will be blocked for further use.

A training video for the correct use of the LactoSens® Test Kit can be found at *www.lactosens.com*.

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References

American Dairy Products Institute (Hg.) (2002): Ingredient Description Brochure. Online verfügbar unter https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwiTiNDzobvMAhXMOhQKHX2WBBIQFggdMAA&url=https%3A%2F%2Fwww.adpi.org%2FPortals%2F0%2FPDF%2FPublications%2FIngredient%2520Descprition%2520Brochure-Revised012203.pdf&usg=AFQjCNHx-zQYlH2O6yLwD5iCZZrsmL-Iwg&sig2=c8lADcyX-sjCUigz-t_LnA, zuletzt geprüft am 02.02.2016.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2010): Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia. Parma.

Essig, A. M.; Kleyn, D. H. (1983): Determination of lactose in milk: comparison of methods. In: Journal - Association of Official Analytical Chemists 66 (6), S. 1514–1516.

Fiechter, A.; Aiba, S.; Atkinson, B.; Böing, J.; Bylinkina, E.; Dellweg, H. et al. (Hg.) (1980): Advances in Biochemical Engineering, Volume 15. Berlin, Heidelberg: Springer Berlin Heidelberg (Advances in Biochemical Engineering/Biotechnology).

Folin, O; Denis, W (1918): The determination of lactose in milk. In: The Journal of Biological Chemistry (33), S. 521–524.

Global Lactose-free Food market 2016-2020 (2016): Research and Markets (Report No. 3704564).

Harju, M.; Kallioinen, H.; Tossavainen, O. (2012): Lactose hydrolysis and other conversions in dairy products. Technological aspects. In: International Dairy Journal 22 (2), S. 104–109. DOI: 10.1016/j.idairyj.2011.09.011.

Ingram, Catherine J. E.; Mulcare, Charlotte A.; Itan, Yuval; Thomas, Mark G.; Swallow, Dallas M. (2009): Lactose digestion and the evolutionary genetics of lactase persistence. In: Hum Genet 124 (6), S. 579–591. DOI: 10.1007/s00439-008-0593-6.

Järvelä, Irma; Torniainen, Suvi; Kolho, Kaija-Leena (2009): Molecular genetics of human lactase deficiencies. In: Annals of Medicine 41 (8), S. 568–575. DOI: 10.1080/07853890903121033.

Jelen, Paul; Tossavainen, Olli (2003): Low lactose and lactose-free milk and dairy products - prospects, technologies and applications. suppl. IDF 2nd World Symposium of Dairy Products in Human Health. In: Australian Journal of Dairy Technology 58 (2), S. 161–165.

Kleyn, Dick H. (1985): Determination of Lactose by an Enzymatic Method. In: Journal of dairy science 68 (10), S. 2791–2798. DOI: 10.3168/jds.S0022-0302(85)81167-X.

Kouaouci, Rachid (2007): Analytical Methods for Lactose Quantification in Milk and Milk Products. Centre d'expertise en production laitière du Québec. IDF International Symposium -Lactose and its Derivatives. Moscow, 2007.

Mahoney, Raymond R (1998): Galactosyl-oligosaccharide formation during lactose hydrolysis: a review. In: Food Chemistry 63 (2), S. 147–154.

National Dairy Council (1993): Newer knowledge of milk and other fluid dairy products. Rosemont, IL: National Dairy Council.

National Institute of Diabetes and Digestive and Kidney Diseases (Hg.) (2014): Lactose Intolerance. U.S. Department of Health and Human Services. Online verfügbar unter http://www.niddk.nih.gov/health-information/health-topics/digestive-diseases/lactose-intolerance/Pages/facts.aspx, zuletzt geprüft am 02.05.2016.

Neelakatnan, S; Mohanty, A K; Kaushik, Jai K (1999): Production and use of microbial enzymes for dairy processing. Fermentation - Science & Technology. In: Current Science 77 (1), S. 143–148.

Perati, Pranathi; De Borba, Brian; Rohrer, Jeffrey (2015): Determination of Lactose in Lactose-Free Milk Products by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. Application Note 248. Sunnyvale, CA.

Suchy, Frederick J.; Brannon, Patsy M.; Carpenter, Thomas O.; Fernandez, Jose R.; Gilsanz, Vicente; Gould, Jeffrey B. et al. (2010): National Institutes of Health Consensus Development Conference: lactose intolerance and health. In: Annals of internal medicine 152 (12), S. 792–796. DOI: 10.7326/0003-4819-152-12-201006150-00248.

U.S. National Library of Medicine (2016): LCT - Lactase. Bethesda, MD. Online verfügbar unter https://ghr.nlm.nih.gov/gene/LCT.

Vesa, Tuula H.; Marteau, Philippe; Korpela, Riitta (2000): Lactose Intolerance. In: Journal of the American College of Nutrition 19 (sup2), 165S-175S. DOI: 10.1080/07315724.2000.10718086.

Woychik, J H; Holsinger, V H (1977): Use of Lactase in the Manufacture of Dairy Products. In: Robert L. Ory und Allen J. St. Angelo (Hg.): Enzymes in Food and Beverage Processing, Bd. 47. WASHINGTON, D. C.: AMERICAN CHEMICAL SOCIETY (ACS Symposium Series), S. 67–79.

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Simple

- Easy sample preparation - 1:5 dilution
- Simple handling
- Very little training

Fast

- Instant results - less than 1 minute measuring time
- Fast product release
- Optimization of production process and capacities

Accurate

- Very low quantitation limit - 0.008%
- High accuracy - 90-110% recovery
- Excellent precision - less than 5% standard deviation
- In house quality control
- Fast product release
- Traceability
- Documentation

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