

NordVal International Certificate

Issued for:	LactoSens®R and LactoSens®R for NOLA™ Fit
NordVal No:	048
First approval date:	05 February 2018
Extension date:	22 October 2021
Renewal date:	25 October 2023
Valid until:	01 November 2025

LactoSens®R and LactoSens®R for NOLA™ Fit

Manufactured by:

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fulfils the requirements of the NordVal Validation Protocol 2.

The method validation was carried out by the Austrian Agency for Health and Food Safety (AGES). The results obtained are satisfactory and document that there is no statistical difference in the performances between the methods for determination of lactose in milk and milk products (white mass and flavoured products) for levels in the quantitation range 0.008% - 0.2%.

The performance of the LactoSens®R assay was additionally validated to demonstrate conformity with AOAC SMPR 2018.009 "Standard Method Performance Requirements (SMPR) for Lactose in Low-Lactose or Lactose-Free Milk, Milk Products, and Products Containing Dairy Ingredients". LactoSens®R was compared against the method "High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)". LactoSens®R is adopted as an AOAC method, AOAC 2020.01, and published in J AOAC Int. 2020;103(6):1534-1546.

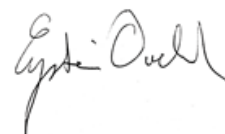
DirectSens is certified for LactoSens® biosensor production with ISO 9001:2015 by TÜV NORD Austria.

Date: 25 October 2023

Yours sincerely,



Hrólfur Sigurðsson
Chair of NordVal International



Eystein Oveland
NMKL Executive Director



PRINCIPLE OF THE METHOD

DirectSens® has developed a biosensor for the determination of residual lactose in lactose-free milk products. An enzyme immobilized on a disposable test strip can detect lactose directly and accurately using the LactoSens® Reader with sensors from the LactoSens®R Biosensor Assay Kit (LK1225) and LactoSens®R for NOLA™ Fit Biosensor Assay Kit (LK1325). The electrochemical measurement is based on a principle called amperometry. The highly specific enzyme on the biosensor oxidizes the lactose molecules in the sample. Resulting electrons are conducted to the LactoSens® Reader and the software transfers the analytical signal into a lactose concentration according to the calibration function. Samples with a fat content ≥ 20 % must be centrifuged for 10 min. at 10,000 rpm, analysis performed on the aqueous phase, and the reading result multiplied with the dilution factor.

The LactoSens®R Biosensor Assay Kit (LK1225) can be used for determination of residual lactose in dairy products where yeast neutral lactase is used for hydrolysis and LactoSens®R for NOLA™ Fit Biosensor Assay Kit (LK1325) can be used for determination of residual lactose in dairy products where bifido bacterial lactase (e.g. NOLA™ Fit) is used for hydrolysis.

LactoSens®R and LactoSens®R for NOLA™ Fit perform satisfactorily for lactose levels of 0.008 % to 0.2 % and samples with lactose levels above 0.2 % can be analysed when diluted with water prior to buffer dilution.

FIELD OF APPLICATION

Milk and milk products hydrolysed with yeast neutral or bifido bacterial lactase. Validation only for cow/bovine milk products.

HISTORY

In 2019, the certificate for LactoSens® 0.01% method was extended to include milk samples with a fat content up to 36 % and fermented milk products; yoghurt, cream, sour cream and curd. Further, it was evaluated that the sensor also is applicable to lactose-free flavoured yoghurts and milk drinks (coffee, cacao, fruits). Finally, it was evaluated that the sensors are stable for at least 3 days storage at 30 °C. By the time of extension, the name of the method was changed to LactoSens®R.

In 2021, the certificate for LactoSens®R was extended to include determination of the lactose levels in bifido bacterial lactase (e.g. NOLA™ Fit) treated milk, cream and yoghurt samples using the LactoSens®R for NOLA™ Fit Biosensor Assay Kit.

METHOD COMPARISON STUDY

The validation study was carried out by the expert laboratory AGES. LactoSens®R and LactoSens®R for NOLA™ Fit were compared against the reference method “High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)” [1]. Unless otherwise stated, the measurement has been performed five times on the same sample.

Ruggedness

The ruggedness of the method was tested by repeatedly analysing samples with lactose (**Table 1**) at various:

- measurement temperatures (20 °C and 24 °C)
- sample temperatures (4 °C, 20 °C and 40 °C)
- fat content of the milk (0.8 – 36 %)
- pH and texture

Table 1. The samples tested, their pH range and fat content

Sample	pH range	Fat content (%)
Milk/Cream		0.8 - 36
Yoghurt	4.4 - 4.6	0.1 - 3.5
Sour cream	4.3 - 4.4	15
Curd	3.8 - 3.9	20

The obtained relative standard deviations, RSD, of the measurements were no more than 9 % and the recoveries were 91 % and above. The results of the extension studies, documenting that the LactoSens®R and LactoSens®R for NOLA™ Fit methods are applicable to milk samples with a fat content up to 36 % and fermented milk products; yoghurt, cream, sour cream and curd, are given in **Table 2** and **Table 3**.

Table 2. The samples and results of yoghurt, cream, sour cream and curd for LactoSens®R

Sample	Fat content	pH	Alternative method				Reference method (% lactose)
			Mean (% lactose)	SD (%)	RSD (%)	Recovery (%)	
Cream	10	-	<0.008	-	-	-	<0.008
	10	-	0.066	0.002	4	99	0.066
	22	-	<0.008	-	-	-	<0.008
	22	-	0.060	0.001	2	93	0.064
	36	-	<0.008	-	-	-	<0.008
	36	-	0.068	0.002	3	95	0.071
Yoghurt	0.1	4.4	0.011	0.001	9	98	0.014
	0.1	4.5	0.033	0.001	3	108	0.031
	1	4.5	0.046	0.004	9	101	0.046
	1.8	4.6	0.013	0.001	9	98	0.014
	2.5	4.5	<0.008	-	-	-	<0.008
	3.5	4.6	0.092	0.003	3	108	0.085
Sour cream	15	4.3	<0.008	-	-	-	<0.008
	15	4.4	<0.008	-	-	-	<0.008
	15	4.3	<0.008	-	-	-	<0.008
Curd	20	3.9	<0.008	-	-	-	<0.008
	20	3.8	<0.008	-	-	-	<0.008
	20	3.9	<0.008	--	-	-	<0.008

Table 3. The samples and results of milk, yoghurt and cream for LactoSens®R for NOLA™ Fit

Sample	(%) lactose by reference method	N	Mean (%) lactose by LactoSens®R for NOLA™ Fit	RSD (%)	Recovery (%)
Milk (fat 3.5 %)	<0.008	5	<0.008	N/A	100
	0.013	5	0.013	3	100
	0.077	5	0.078	3	101
	0.163	5	0.170	4	104
	0.318*	5	0.335	1	105
Yoghurt (fat 3.5 %)	<0.008	5	<0.008	N/A	100
	0.013	5	0.012	6	92
	0.056	5	0.052	6	93
	0.156	5	0.158	3	101
	0.545*	5	0.544	1	100
Cream (fat 36 %)	<0.008	5	<0.008	N/A	100
	0.011	5	0.010	8	91
	0.060	5	0.059	6	98
	0.109	5	0.099	6	91
	0.404*	5	0.434	1	107

* Samples with lactose concentration outside of the quantitation range (0.008 - 0.2 %) have been diluted 1:5 with water (1 part sample plus 4 parts water) followed by standard dilution 1:2 (1 part milk plus 1 part dilution buffer).

The results showed that the method is rugged for the parameters tested.

Specificity

In order to check for interferences, samples with lactose content of 0.022 % were spiked with different sugars; glucose, galactose, sucrose and fructose. The contents of the spiked sugars were 1 % and 2 %, respectively. The study showed that there were no cross-reactivity interferences from the added sugars.

Further, the specificity was tested for vitamins. Milk samples, with lactose content of less than 0.010 %, were fortified with Vitamin A (5 mg/L), Vitamin D (0.045 mg/L), Vitamin B6 (12 mg/L) and Vitamin E (60 mg/L). The results showed that the vitamins did not influence on the measurement of lactose content.

Interferences from flavours and added fruit preparations were also tested. The tested flavours were coffee, vanilla, cocoa at concentrations 0.3 -1 % and banana, peach/apricot, strawberry and cherry at concentrations 17 or 18 %. No interferences were observed from flavours or fruit preparations.

Stability testing and batch-to-batch variation

Sensors stored at 4 °C regularly measured with the samples containing various concentration of lactose showed that the sensors are stable for at least 12 months.

During production of LactoSens®R biosensors every critical step is strictly monitored, and the final product is tested according to quality control requirements. Every batch of biosensors is factory calibrated to ensure absolutely repeatability of lactose determination between the batches.

Storage at 30°C for three days was tested and showed recoveries of 95 - 100 %, indicating that sensors are stable for at least 3 days at 30 °C.

Limit of quantification (LOQ)

The minimum level at which lactose can be quantified with acceptable precision and recovery was determined by using the reference method and the alternative method, analysing different levels of lactose. The results are given in **Table 4**.

Table 4. Determination of LOQ

Sample	Reference method		Alternative method		
	N	Mean ± SD (%)	N	Mean ± SD (%)	Recovery (%)
1	2	<0.005	5	<0.008	-
2	2	0.0077 ± 0.002	5	0.008 ± 0.000	104
3	2	0.0088 ± 0.001	5	0.008 ± 0.001	91
4	2	0.011 ± 0.002	20	0.012 ± 0.001	109
5	1	0.012	5	0.011 ± 0.001	92
6	2	0.0225 ± 0.002	5	0.022 ± 0.002	96
7	2	0.032 ± 0.005	5	0.031 ± 0.003	97
8	2	0.062 ± 0.006	5	0.064 ± 0.003	103

N = number of replicates

The lowest level tested with satisfactory precision (SD) and accuracy (recovery) is 0.008 % lactose content. Values below 0.0075 % are displayed as <0.008 % by the LactoSens® software. All other values are shown as in the table.

The lowest level tested with satisfactory precision (SD) and accuracy (recovery) is 0.008 % lactose content. Samples with lactose levels of 0.5 % which is above the upper LOQ have also been tested in comparison to the reference method. Those samples have been diluted with water prior to standard dilution with buffer. Lactose results displayed in the LactoSens® software have been multiplied with the additional dilution factor.

Reliability: precision – internal reproducibility

The reliability of the method was tested by including milk samples at different concentration levels. Each sample was measured 5 times and repeated on 3 different days. The relative standard deviation of the repeatability, RSD, for each day and the relative standard deviation of the internal standard deviation RSDIR for the results obtained at different days along with the HorRat values were calculated. A HorRat value of no more than 2 is considered acceptable.

Table 5 shows a summary of the results.

Table 5. Results of 3 different samples with different lactose concentrations levels over 3 days

Sample (% lactose)	Day	Mean (%)	SD (%)	RSD (%)	Mean (%)	RSD _{IR} (%)	HorRat	Recovery (%)
Milk (0.023)	1	0.022	0.001	5.93	0.022	6.95	0.97	98
	2	0.023	0.001	3.82				
	3	0.022	0.002	9.44				
Milk (0.062)	1	0.064	0.003	4.69	0.063	6.61	1.10	102
	2	0.061	0.005	8.99				
	3	0.064	0.003	4.37				
Milk (0.128)	1	0.132	0.007	5.10	0.128	4.85	0.88	103
	2	0.128	0.005	4.16				
	3	0.137	0.002	1.73				

The results show that the precision as well as the accuracy are satisfactory.

INTERMEDIATE STUDY

An intermediate study was performed at a second independent expert laboratory: Landwirtschaftliches Zentrum Baden-Württemberg – Milchwirtschaft Wangen (Germany). Samples of milk, yoghurt, sour cream and curd at different levels were analysed. The results are given in **Table 6**.

Table 6. Results of the intermediate study

Sample	(%) lactose by reference method	N	Mean (%) lactose by LactoSens®R	RSD (%)	Recovery (%)
Milk	0.005	5	<0.008	-	-
	0.011	5	0.012	6	109
	0.023	5	0.021	6	91
Yoghurt	0.085	3	0.083	3	97
	0.028	3	0.031	3	91
	<0.008	3	<0.008	-	-
Sour cream	<0.008	3x3	<0.008	-	-
Curd	<0.008	3x3	<0.008	-	-

CONCLUSION

The validation study shows that the LactoSens®R and LactoSens®R for NOLA™ Fit perform satisfactorily for lactose levels of 0.008 % to 0.2 % with equivalent results to the reference method. Samples with lactose levels above 0.2 % can be analysed when diluted with water prior to buffer dilution.

REFERENCES

1. Fast determination of lactose in dairy products by HPAEC-PAD, Thermo Fisher Scientific Customer Application Note 72633, <https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/can-72633-ic-lactose-dairy-products-can72633-en.pdf>