Lactose/D-Glucose

UV-method

for the determination of lactose and D-glucose in foodstuffs and other materials

BOEHRINGER MANNHEIM / R-BIOPHARM
Enzymatic BioAnalysis / Food Analysis

For use in foodstuff hygiene only. **Store at 2-8°C**

Cat. No. 10 986 119 035

Test-Combination for 32 determinations each

For recommendations for methods and standardized procedures see references (A2, B2)

Principle (Ref. 1)

Lactose is hydrolyzed at pH 6.6 to D-glucose and D-galactose in the presence of the enzyme β-galactosidase and water (1).

(1) Lactose + H_2O $\xrightarrow{\beta\text{-galactosidase}}$ D-glucose + D-galactose

D-Glucose is phosphorylated at pH 7.6 by the enzyme hexokinase (HK) and adenosine-5'-triphosphate (ATP) to D-glucose-6-phosphate (G-6-P) with the simultaneous formation of adenosine-5'-diphosphate (ADP) (2).

(2) D-Glucose + ATP $\xrightarrow{HK}$ G-6-P + ADP

In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) to D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (3).

(3) G-6-P + $NADP^+$ $\xrightarrow{G6P\text{-}DH}$ D-gluconate-6-phosphate + NADPH + H^+

The amount of NADPH formed in reaction (3) is stoichiometric to the amount of D-glucose and lactose, resp.. NADPH is measured by its light absorbance at 334, 340 or 365 nm.

The amount of lactose is calculated from the difference between the D-glucose concentration with and without hydrolysis with β-galactosidase.

The Test-Combination contains

1. Bottle 1 with approx. 600 mg lyophilizate, consisting of: citrate buffer, pH approx. 6.6
2. Bottle 2 with approx. 1.7 ml suspension β-galactosidase, approx. 100 U
3. Two bottles 3 with approx. 5 g powder mixture, each, consisting of: triethanolamine buffer, pH approx. 7.6; NADP, approx. 70 mg; ATP, approx. 170 mg; magnesium sulfate
4. Bottle 4 with approx. 1.4 ml suspension, consisting of: hexokinase, approx. 400 U; glucose-6-phosphate dehydrogenase, approx. 200 U

Preparation of solutions

1. Dissolve contents of bottle 1 with 7.0 ml redist. water.
2. Use suspension of bottle 2 undiluted.
3. Dissolve contents of one bottle 3 with 32 ml redist. water.
4. Use suspension of bottle 4 undiluted.

Stability of reagents

The contents of bottle 1 are stable at 2-8°C (see pack label).
Solution 1 is stable for 3 months at 2-8°C.
Bring solution 1 to 20-25°C before use.
The contents of bottles 2 and 3 are stable at 2-8°C (see pack label).
Solution 3 is stable for 4 weeks at 2-8°C or for 2 months at −15 to −25°C.
Bring solution 3 to 20-25°C before use.
The contents of bottle 4 are stable at 2-8°C (see pack label).

Procedure

Wavelength[1]: 340 nm, Hg 365 nm or Hg 334 nm
Glass cuvette[2]: 1.00 cm light path
Temperature: 20-25°C
Final volume: 3.270 ml
Read against air (without a cuvette in the light path) or against water
Sample solution: 4-200 µg lactose and D-glucose/assay[3] (in 0.100-0.500 ml sample volume)

Pipette into cuvettes	Blank lactose sample	Lactose sample	Blank D-glucose sample	D-Glucose sample
solution 1*	0.200 ml	0.200 ml	-	-
suspension 2	0.050 ml	0.050 ml	-	-
sample solution**	-	0.100 ml	-	0.100 ml
Mix*, incubate for minimum 20 min at 20-25°C. Add:				
solution 3	1.000 ml	1.000 ml	1.000 ml	1.000 ml
redist. water	2.000 ml	1.900 ml	2.250 ml	2.150 ml
Mix***, read absorbances of the solutions after approx. 2 min (A_1). Start reaction by addition of:				
suspension 4	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix***, wait for completion of the reaction (approx. 15 min) and read absorbances of the solutions (A_2). If the reaction has not stopped after 15 min, continue to read the absorbances at 2 min intervals until the absorbances increases constantly for 2 min.				

* Pipette solution 1, suspension 2 and sample solution, each onto the bottom of the cuvette and mix by gentle swirling. When using a plastic spatula, remove it from the cuvette only directly before measuring absorbance A_1.

** Rinse the enzyme pipette or the pipette tip of the piston pipette with sample solution before dispensing the sample solution.

*** For example, with a plastic spatula or by gentle swirling after closing the cuvette with Parafilm (trademark of the American Can Company, Greenwich, Ct., USA)

If the absorbance A_2 increases constantly, extrapolate the absorbance to the time of the addition of suspention 4 (HK/G6P-DH).

Determine the absorbance differences (A_2-A_1) for both, blanks and samples. Subtract the absorbance difference of the blank from the absorbance difference of the corresponding sample.

$$\Delta A = (A_2\text{-}A_1)_{sample} - (A_2\text{-}A_1)_{blank}$$

It follows $\Delta A_{D\text{-glucose}}$ (from "D-glucose sample") and
$\Delta A_{lactose\ +\ D\text{-glucose}}$ (from "lactose sample")
The difference of these values stands for $\Delta A_{lactose}$.

The measured absorbance differences should, as a rule, be at least 0.100 absorbance units to achieve sufficiently precise results (see "Instructions for performance of assay" and "Sensitivity and detection limit", pt. 4).

Calculation

According to the general equation for calculating the concentration:

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \text{ [g/l]}$$

V = final volume [ml]

v = sample volume [ml]

MW = molecular weight of the substance to be assayed [g/mol]

d = light path [cm]

ε = extinction coefficient of NADPH at
340 nm = 6.3 [l × $mmol^{-1}$ × cm^{-1}]
Hg 365 nm = 3.5 [l × $mmol^{-1}$ × cm^{-1}]
Hg 334 nm = 6.18 [l × $mmol^{-1}$ × cm^{-1}]

It follows for lactose (calculated as anhydrous lactose):

$$c = \frac{3.270 \times 342.3}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A = \frac{11.19}{\varepsilon} \times \Delta A_{lactose} \text{ [g lactose/l sample solution]}$$

for lactose (calculated as lactose-monohydrate):

$$c = \frac{3.270 \times 360.32}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A_{D\text{-lactose}} = \frac{11.78}{\varepsilon} \times \Delta A_{lactose}$$

[g lactose × H_2O/l sample solution]

1 The absorption maximum of NADPH is at 340 nm. On spectrophotometers, measurements are taken at the absorption maximum; if spectralline photometers equipped with a mercury vapor lamp are used, measurements are taken at a wavelength of 365 nm **or** 334 nm.

2 If desired, disposable cuvettes may be used instead of glass cuvettes.

3 See instructions for performance of assay

0917.**11748319001**®

for D-glucose:

$$c = \frac{3.270 \times 180.16}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A_{D\text{-glucose}} = \frac{5.891}{\varepsilon} \times \Delta A_{D\text{-glucose}}$$

[g D-glucose/l sample solution]

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor F.

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the result is to be calculated from the amount weighed:

$$\text{Content}_{\text{lactose}} = \frac{c_{\text{lactose}}[\text{g/l sample solution}]}{\text{weight}_{\text{sample}} \text{ in g/l sample solution}} \times 100 \text{ [g/100 g]}$$

$$\text{Content}_{\text{D-glucose}} = \frac{c_{\text{D-glucose}}[\text{g/l sample solution}]}{\text{weight}_{\text{sample}} \text{ in g/l sample solution}} \times 100 \text{ [g/100 g]}$$

1. Instructions for performance of assay

The amout of lactose and D-glucose present in the assay has to be between 10 µg and 200 µg (measurement at 365 nm) or 4 µg and 100 µg (measurement at 340, 334 nm), respectively. In order to get a sufficient absorbance difference, the sample solution is diluted to yield a lactose and D-glucose concentration between 0.3 and 2 g/l or 0.2 and 1 g/l respectively.

Dilution table

Estimated amount of lactose and D-glucose per liter		Dilution with water	Dilution factor F
measurement at			
340 or 334 nm	365 nm		
< 1.0 g	< 2.0 g	-	1
1.0-10.0 g	2.0-20.0 g	1 + 9	10
10.0-100 g	20.0-200 g	1 + 99	100
> 100 g	> 200 g	1 + 999	1000

If the measured absorbance difference (ΔA) is too low (e.g. < 0.100), the sample solution should be prepared again (weigh out more sample or dilute less strongly) *or* the sample volume to be pipetted into the cuvette can be increased up to 0.500 ml (neutralize sample in advance, if necessary). The volume of water added after incubation must then be reduced so as to obtain the same final volume in the assays for sample and blank. The new sample volume v must be taken into account in the calculation.

2. Technical information

2.1 Solution 1 and the enzyme suspension 2 are pipetted (e.g. with a piston-type pipette) onto the bottom of the cuvette. Subsequently the sample solution is pipetted onto the surface of the solution in the cuvette (and not onto the wall of the cuvette). (One must not be afraid of a contamination, because a new pipette (tip) is used for the next sample solution.)

2.2 Jerky swinging or swirling of the cuvette proved to be best when mixing small volumes (e.g. 0.350 ml) in the cuvette.

2.3 The calculation of the results can be done on the basis of anhydrous lactose (molecular weight 342.3), which can be recommended, or on the basis of lactose monohydrate (molecular weight 360.32). When comparing data, one has to make sure that the same molecular weight has been used for the calculation (figures for lactose monohydrate are 5% higher than those for anhydrous lactose).

3. Specificity

The method is specific for lactose and D-glucose.

In the analysis of commercial lactose monohydrate (molecular weight 360.32) and of D-glucose (water-free; molecular weight 180.16), resp. D-glucose monohydrate (molecular weight 198.17), results of < 100 % have to be expected because the materials absorb moisture.

4. Sensitivity and detection limit

The smallest differentiating absorbance for the procedure is 0.005 absorbance units. This corresponds to a maximum sample volume v = 0.500 ml and measurement at 340 nm of a lactose concentration of 2 mg/l, resp. 1 mg D-glucose/l sample solution (if v = 0.100 ml, this corresponds to 10 mg lactose/l, resp. 5 mg D-glucose/l sample solution).

The detection limit of 7 mg lactose/l, resp. 4 mg D-glucose/l is derived from the absorbance difference of 0.020 (as measured at 340 nm) and a maximum sample volume v = 0.500 ml.

5. Linearity

Linearity of the determination exists from approx. 4 µg lactose + D-glucose/ assay (7 mg lactose + D-glucose/l sample solution; sample volume v = 0.500 ml) to 200 µg lactose + D-glucose/ assay (2 g lactose + D-glucose/l sample solution; sample volume v = 0.100 ml).

6. Precision

In a double determination of D-glucose using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. With a sample volume of v = 0.100 ml and measurement at 340 nm, this corresponds to a D-glucose concentration of approx. 5-10 mg/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor F. If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 10 g/l, a difference of 0.05-0.1 g/100 g can be expected.)

In a double determination of lactose using one sample solution, a difference of 0.010 to 0.015 absorbance units may occur in the presence of D-glucose in the sample. With a sample volume of v = 0.100 ml and measurement at 340 nm, this corresponds to a lactose concentration of approx. 15-25 mg/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor F. If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 10 g/l, a difference of 0.15-0.25 g/ 100 g can be expected.)

The following data for the determination of lactose via D-glucose have been published in the literature:

Ice-cream mix:
x = 12.814 g /100 g r = 0.652 g/100 g R = 0.905 g/100 g
x = 13.371 g/100 g r = 0.291 g/100 g R = 1.112 g/100 g

Skin milk powder:
x = 51.046 g /100 g r = 1.150 g/100 g R = 2.306 g/100 g
x = 51.670 g/100 g r = 1.082 g/100 g R = 2.495 g/100 g

For further data see references (Ref. A2.7)

As the working technique is the same, the procedures differ only by the start enzymes and the coenzymes, the data for the determination of lactose via D-galactose may also be used for the evaluation of measurements of lactose via D-glucose.

Lactose in chocolate:
x = 6.01 g/100 g s = 0.04 g/100 g CV = 0.7 % (Ref. B1.1)

85 µg lactose/assay CV = 1.13 %
170 µg lactose/assay CV = 0.78 % (Ref. B1.2)

Lactose in milk and milk products:
r = 0.05 × (content$_{\text{lactose}}$ in g/100 g) g/100 g
R= 0.06 × (content$_{\text{lactose}}$ in g/100 g) g/100 g

Lactose in chocolate:
r = 0.188 g/100 g $s_{(r)} = \pm 0.066$ g/100 g
R= 0.434 g/100 g $s_{(R)} = \pm 0.153$ g/100 g

For further data see references (Ref. B2.5)

7. Recognizing interference during the assay procedure

7.1 If the conversion of D-glucose has been completed according to the time given under "Procedure", it can be concluded in general that no interference has occurred.

7.2 On completion of the reaction, the determination can be restarted by adding D-glucose (qualitative or quantitative): if the absorbance is altered subsequent to the addition of the standard material, this is also an indication that no interference has occurred.

The reaction cannot be restarted with lactose as, subsequent to altering the reaction conditions from pH 6.6 to pH 7.6 ("change of the buffer"), lactose is no longer cleaved.

7.3 Operator error or interference of the determination through the presence of substances contained in the sample can be recognized by carrying out a double determination using two different sample volumes (e.g. 0.100 ml and 0.200 ml): the measured differences in absorbance should be proportional to the sample volumes used.

When analyzing solid samples, it is recommended that different quantities (e.g. 1 g and 2 g) be weighed into 100 ml volumetric flasks. The absorbance differences measured and the weights of sample used should be proportional for identical sample volumes.

The use of "single" and "double" sample volumes in double determinations is the simplest method of carrying out a control assay in the determination of lactose.

7.4 Possible interference caused by substances contained in the sample can be recognized by using an internal standard as a control: in addition to the sample, blank and standard determinations, a further determination should be carried out with sample **and** assay control solution in the same

assay. The recovery can then be calculated from the absorbance differences measured.

7.5 Possible losses during the determination can be recognized by carrying out recovery tests: the sample should be prepared and analyzed with and without added standard material. The additive should be recovered quantitatively within the error range of the method.

8. Reagent hazard

The reagents used in the determination of lactose and D-glucose are not hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulation 67/548/EEC and subsequent alteration, supplementation and adaptation guidelines. However, the general safety measures that apply to all chemical substances should be adhered to.

After use, the reagents can be disposed of with laboratory waste, but local regulations must always be observed. Packaging material can be disposed of in waste destined for recycling.

9. General information on sample preparation

In carrying out the assay:
Use **clear, colorless and practically neutral liquid samples** directly, or after dilution according to the dilution table, and of a volume up to 0.500 ml;
Filter **turbid solutions**;
Degas **samples containing carbon dioxide** (e.g. by filtration);
Treat **"strongly colored" samples** that are used undiluted or with a higher sample volume with polyvinylpolypyrrolidone (PVPP) or with bentonite, e.g. 1 g/100 ml;
Crush or homogenize **solid or semi-solid samples**, extract with hot water or dissolve in water and filter if necessary; resp. remove turbidities or dyestuffs by Carrez clarification;
Deproteinize **samples containing protein** with perchloric acid or with trichloroacetic acid; alternatively clarify with Carrez reagents;
Extract **samples containing fat** with hot water (extraction temperature should be above the melting point of the fat involved). Cool to allow the fat to separate, make up to the mark, place the volumetric flask in an ice bath for 15 min and filter; alternatively clarify with Carrez-solutions after the extraction with hot water;
Break up **emulsions** with trichloroacetic acid.

Carrez clarification:
Pipette the liquid sample into a 100 ml volumetric flask which contains approx. 60 ml redist. water, or weigh sufficient quantity of the sample into a 100 ml volumetric flask and add approx. 60 ml redist. water. Subsequently, carefully add 5 ml Carrez-I-solution (potassium hexacyanoferrate(II) (ferrocyanide), 85 mM = 3.60 g $K_4[Fe(CN)_6] \times 3\ H_2O$/100 ml) and 5 ml Carrez-II-solution (zinc sulfate, 250 mM = 7.20 g $ZnSO_4 \times 7\ H_2O$/100 ml). Adjust to pH 7.5-8.5 with sodium hydroxide (0.1 M; e.g. 10 ml). Mix after each addition. Fill the volumetric flask to the mark, mix and filter.

Samples containing protein should only be deproteinized with perchloric acid or with trichloroacetic acid in the absence of sucrose and maltose as these disaccharides are fully or partically hydrolized with the release of D-glucose. The Carrez-clarification is recommended for normal use.

10. Application examples

Determination of lactose in milk, skimmed milk, butter-milk and whey
Accurately weigh approx. 1 g sample into a 100 ml volumetric flask and add 60 ml water. For clarification add 5 ml Carrez-I-solution (3.60 g potassium hexacyanoferrate(II), $K_4[Fe(CN)_6] \times 3\ H_2O$/100 ml), 5 ml Carrez-II-solution (7.20 g zinc sulfate, $ZnSO_4 \times 7\ H_2O$/100 ml) and 10 ml NaOH (0.1 M), mix after each addition, fill up to the mark with water, mix and filter. Use the clear, possibly slightly turbid solution for the assay, diluted, if necessary.

Determination of lactose and D-glucose in sweetened condensed milk and ice cream
Accurately weigh approx. 1 g sample into a 100 ml volumetric flask, add approx. 60 ml water and keep at approx. 70°C for 15 min. Shake from time to time. For clarification add 5 ml Carrez-I-solution (3.60 g potassium hexacyanoferrate(II), $K_4[Fe(CN)_6] \times 3\ H_2O$/ 100 ml), 5 ml Carrez-II-solution (7.20 g zinc sulfate, $ZnSO_4 \times 7\ H_2O$/ 100 ml) and 10 ml NaOH (0.1 M) mix after each addition, allow to cool to 20-25°C, fill up to the mark with water, mix and filter. Use the clear, possibly slightly opalescent solution for the assay, diluted according to dilution table, if necessary.

Determination of lactose in margarine and butter
Accurately weigh approx. 5 g of homogeneous sample into a beaker, add approx. 70 ml redist. water and heat while stirring on a heatable magnetic stirrer until the fat melts. Allow to cool in an ice-bath and transfer the aqueous phase into a 100 ml volumetric flask by pipetting. Repeat extraction with approx. 20 ml redist. water. Allow to warm up to 20-25°C and fill up to the mark with redist. water. Place the volumetric flask in an ice-bath or into a refrigerator for 15 min. Filter through a fluted filter paper. Use filtrate diluted according to the dilution table, if necessary, for the assay.

Determination of lactose and D-glucose in hard cheese or chocolate
Grate cheese crumbs or chocolate, accurately weigh approx. 2 g into a 100 ml volumetric flask, add about 70 ml water and incubate for 15 min at approx. 70°C. Shake frequently. After cooling to 20-25°C, make up with water to 100 ml and mix. To separate the fat, place in a refrigerator for approx. 20 min and filter. Discard the first few ml. Use the clear, possibly slightly opalescent solution for the assay.

Determination of lactose and D-glucose in instant baby food, restorative food, ice cream (see pt. 9)
Accurately weigh approx. 1 g of sample into a 100 ml volumetric flask, add about 60 ml water and incubate for 15 min at approx. 70°C, shake from time to time. After cooling to 20-25°C, add 10 ml perchloric acid (3 M) and mix. After 10 min neutralize with KOH (3 M) to pH 7-8, fill up to the mark with water, mix and place in a refrigerator for 20 min to precipitate the $KClO_4$ formed, filter. Use the clear, possibly slightly turbid solution for the assay, diluted, if necessary.

Determination of lactose and D-glucose in meat sausage and meat loafs
Accurately weigh approx. 5 g of cut or grated sample (mixer) into a 100 ml volumetric flask, add about 70 ml of water and incubate for 15 min at approx. 70°C. Allow to cool to 20-25°C, fill up with water to the mark and mix. For separation of fat, place in a refrigerator for 20 min and filter. Use the clear, possibly slightly turbid solution for the assay.

Determination of lactose in yogurt, milk powder and whey powder
Accurately weigh approx. 1 g of sample into a 100 ml volumetric flask, add approx. 60 ml water and keep at approx. 70°C for 15 min. Shake from time to time. For clarification add 5 ml Carrez-I-solution (3.60 g potassium hexacyanoferrate(II), $K_4[Fe(CN_6)] \times 3\ H_2O$/100 ml), 5 ml Carrez-II-solution (7.20 g zinc sulfate, $ZnSO_4 \times 7\ H_2O$/100 ml) and 10 ml NaOH (0.1 M), mix after each addition, allow to cool to 20-25°C, fill up to the mark with water, mix and filter. Use the clear, possibly slightly turbid solution for the assay, diluted according to the dilution table, if necessary.

11. Special preparation of sample for the determination of lactose in the presence of a large excess of D-glucose

The precision of the lactose determination is impaired if the ratio of D-glucose to lactose is higher than e.g. 10:1. In that case the determination should be carried out with the Test-Combination Lactose/D-Galactose, Cat. No. 0 176 303. Alternatively the D-glucose should be as much as possible removed: In the presence of glucose oxidase (GOD) and oxygen from the air D-glucose is oxidized to D-gluconate:

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{D-gluconate} + H_2O_2$$

Hydrogen peroxide is destroyed by catalase:

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

Reagents
Glucose oxidase (GOD) from Aspergillus niger, 200 U/mg (25°C; D-glucose as substrate); amylase and β-fructosidase < 0.01 % each)
Catalase
Triethanolamine hydrochloride, $MgSO_4 \times 7\ H_2O$, NaOH, 4 M

Preparation of solution for 10 determinations
Enzyme solution:
Dissolve 5 mg (≙ approx. 1000 U) GOD in 0.750 ml redist. water, add 325 KU catalase (from bovine liver, 25°C; H_2O_2 as substrate), and mix.
Buffer solution: Dissolve 5.6 g triethanolamine hydrochloride and 0.1 g $MgSO_4 \times 7\ H_2O$ in 80 ml redist. water, adjust to pH 7.6 with NaOH (4 M), and make up to 100 ml with redist. water.

Stability of solutions
The enzyme solution must be prepared freshly daily.
The buffer solution is stable for 4 weeks when stored at 2-8°C.

Performance of D-glucose oxidation

Pipette into a 10 ml volumetric flask	
buffer solution sample solution (up to approx. 0.5% D-glucose) enzyme solution	2.000 ml 5.000 ml 0.100 ml
Pass a current of air (O_2) through the mixture for 1 h; during the oxidation process **check the pH with indicator paper and, if necessary, neutralize the formed acid with NaOH.**	

To inactivite the enzymes GOD and catalase, keep the volumetric flask in a boiling water-bath for 15 min, allow to cool, and dilute to the mark with water. Mix and filter, if necessary. Use the clear solution for the determination of lactose. In a parallel assay, determine the residual D-glucose and consider in the calculation as usual.

12. Further applications

The method may also be used for investigations on pharmaceuticals and for example, when analyzing biological samples.

Determination of lactose and D-glucose in fermentation samples and cell culture media

Place the sample, if necessary after centrifugation, in a water-bath (80°C) for 15 min to stop enzymatic activity. Centrifuge and use the supernatant diluted according to the dilution table, if necessary, for the assay. (Alternatively deproteinization can be carried out by addition of perchloric acid or Carrez-solutions. See afore mentioned examples.)

Homogenize gelatinous agar media with water and proceed as described above.

References

A. References for the determination of lactose and D-glucose

A 1.1 Bahl, R. K. (1971) An enzymatic method for the determination of skimmed milk powder in raw sausages, Analyst **96**,88-92

A 1.2 Bahl, R. K. (1972) An enzymatic method for the determination of lactose in milk including human milk, Analyst **97**, 559-561

A 1.3 Bergmeyer, H.U., Bernt, E., Schmidt, F. & Stork, H. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H.U., Hrsg.) 3. Aufl., Bd. 2, 1241-1246; Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 2nd ed., vol. 3, pp. 1196-1201; Verlag Chemie, Weinheim/Academic Press, Inc., New York and London

A 1.4 Kunst, A., Draeger, B. & Ziegenhorn, J. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., Hrsg.) 3rd ed., vol. VI, pp. 163-172, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel

A 2.1 International Dairy Federation, IDF, International Standard 79:1977, Milk and milk products - Determination of lactose in the presence of other reducing substances

A 2.2 Milch und Milchprodukte - Bestimmung von Lactose in Gegenwart anderer reduzierender Substanzen, Internationaler Standard 79:1977, Milchwissenschaft **33**, 749-751 (1978)

A 2.3 Schweizerisches Lebensmittelbuch, Kapitel 61B (Enzymatische Bestimmungen)/1.5 (1981), Kapitel 1 (Milch)/11.2 (1987), Kapitel 2A (Milchmischgetränke)/09 (1980), Kapitel 9 (Speiseeis)/4.3 (1983)

A 2.4 Nederlandse Norm NEN 3769 (Dezember 1981) Kaas: Fysische en chemische methoden van onderzoek: Enzymatische bepaling van het lactosegehalte, (Cheese - Physical and chemical test methods - Enzymatic determination of the lactose content)

A 2.5 International Dairy Federation, International IDF Standard 79B:1991, Dried Milk, Dried Ice-Mixes & Processed Cheese, Determination of Lactose Content, Enzymatic Methods

A 2.6 Draft International Standard ISO/DIS 5765-1 (1998) Dried milk, dried ice-mixes and processed cheese - determination of lactose content - Part 1: Enzymatic method utilizing the glucose moiety of the lactose

A 2.7 Bulletin of the IDF 285 (1993) Chapter 13, pp. 95 - 105, Milk and milk products - Determination of lactose content - Enzymatic methods

B. References for the determination of lactose and D-galactose

B 1.1 Kurz, G. & Wallenfels, K. (1974) in Methoden der enzymatischen Analyse, (Bergmeyer, H.U., Hrsg.) 3. Aufl., Bd. 2, S. 1225-1229 and S. 1324-1327, Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol. 3, pp. 1180-1184 and pp. 1279-1282, Verlag Chemie, Weinheim/Academic Press, Inc., New York and London

B 1.2 Beutler, H. O. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 3rd ed., vol. VI, pp. 104-112, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel

B 2.1 Schweizerisches Lebensmittelbuch, Kapitel 61B (Enzymatische Bestimmungen)/1.4 (1981), Kapitel 1 (Milch)/1.11.2 (1995), Kapitel 2B (Sauermilchprodukte)/09 (1980), Kapitel 4 (Milchdauerwaren)/5.1 (1993), Kapitel 9 (Speiseeis)/4.3 (1983), Kapitel 22 (Diätetische Lebensmittel und Spezialitätenmittel)/6.3 (1991), Kapitel 28A (Frucht- und Gemüsesäfte u.a.)/5.4 (1988), Kapitel 34 (Gärungsessig)/8.2 (1994)

B 2.2 Gombocz, E., Hellwig, E., Vojir, F. & Petuely, F. (1981) Deutsche Lebensmittel-Rundschau **77,** 3-4 (Galactose) und 10 (Lactose)

B 2.3 Norme Belge - Belgische Norm NBN V 21-023 (1e ,d., juin 1981) Lait et Produits Laitiers: Determination Enzymatique du Lactose, Melk en Zuivelprodukten, Enzymatische Bepaling van Lactose, Milch und Milchprodukte, Enzymatische Bestimmung von Lactose

B 2.4 Deutsche Norm DIN 10344 (August 1982) Bestimmung des Lactose- und Galactosegehalts von Milch und Milchprodukten

B 2.5 Amtliche Sammlung von Untersuchungsverfahren nach § 64. LFGB; Untersuchung von Lebensmitteln: Bestimmung von Lactose in Fleischerzeugnissen (07.00-23/Mai 1983); Bestimmung von Lactose in Wurstwaren (08.00-24/Mai 1983); Bestimmung des Lactose- und Galactosegehalts von Milch und Milchprodukten (01.00-17/September 2010); Bestimmung des Lactose- und Galactosegehalts von Milchprodukten (02.00-9/September 2010); Bestimmung von Lactose in Brot einschließlich Kleingebäck aus Brotteigen (17.00-7/November 1983); Bestimmung von Lactose in feinen Backwaren (18.00-8/November 1984); Bestimmung von Lactose in teiladaptierter Säuglingsnahrung auf Milchbasis (48.01-4/Mai 1985); Bestimmung von Lactose in Kinder-Zwieback und Zwiebackmehl (48.02.07-4/Mai 1985); Bestimmung von Lactose in Schokolade (44.00-6/Dezember 1985)

B 2.6 Österreichisches Lebensmittelbuch (Codex Alimentarius Austriacus), Kapitel B15 (Kakao, Kakaoerzeugnisse, Lebensmittel mit Kakao oder Schokolade, Nougat, Nougatmassen) (1983); Kapitel B22 (Zucker und Zuckerarten) (1983)

B 2.7 OFFICIAL METHODS OF ANALYSIS of the Association of Official Analytical Chemists (Williams, S., ed.) 14th edition (1984), p. 284; 15th edition (1990), vol. 2, pp. 810-811 (984.15)

B 2.8 Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, VDLUFA (Januar 1985) Methodenbuch Band VI, C 20.2.3 (VDLUFA-Verlag Darmstadt)

B 2.9 Niederlande: Warenwet, Uitvoeringsvoorschriften (C II-6) Regeling Onderzoekingsmethoden voor brood; Methode 18: De Bepaling van het Lactosegehalte (Oktober 1986); Dit voorschrift betrijft een methode voor de bepaling van het watervrije lactosegehalte in brood

B 2.10 International Dairy Federation, International IDF Standard 79B:1991: Dried Milk, Dried Ice-Mixes & Processed Cheese, Determination of Lactose Content, Enzymatic Methods

B 2.11 Standard der Russischen Föderation / Standard of the Russian Federation / GOSSTANDART ROSSII GOST R 51259-99 (1999) Milk and milk products. Method for determination of lactose and galactose content

B 2.12 Draft International Standard ISO/DIS 5765-2 (1998) Dried milk, dried ice-mixes and processed cheese - determination of lactose content - Part 2: Enzymatic method utilizing the galactose moiety of the lactose

B 2.13 Bulletin of the IDF 285 (1993) Chapter 13, pp. 95 - 105, Milk and milk products - Determination of lactose content - Enzymatic methods

B 3.1 Essig, A. M. & Kleyn, D. H. (1983) Determination of lactose in milk: comparison of methods, J. Assoc. Off. Anal. Chem. **66**, 1514-1516

B 3.2 Kleyn, D. H., & Trout, J. R. (1984) Enzymatic-ultraviolet method for measuring lactose in milk: collaborative study, J. Assoc. Off. Anal. Chem. **67**, 637-640

B 3.3 Kleyn, D. H. (1985) Symposium: Role and Significance of Enzymes in Dairy Processing; Determination of Lactose by an Enzymatic Method, J. Dairy Sci **68**, 2791-2798

B 3.4 Berner, G. (1970) Zuckerabbau während der Camembert-Reifung. Enzymatische Bestimmung von Lactose, D-Glucose und D-Galactose, Milchwissenschaft **25**. 275-280

B 3.5 Steffen, Chr., Nick, B. & Blanc, B. (1975) Methodik zur enzymatischen Bestimmung von Lactose, Glucose, Galactose und Lactat in Käse. Schweiz. Milchw. Forsch. **4**, 13-15

B 3.6 Salzer, U.-J. (1970) Lactose-Bestimmung in Fleischwaren, Die Fleischwirtschaft **50**, 1229-1232

B 3.7 Bauer, F. & Stachelberger, H. (1984) Ein Schnellnachweis galaktosehaltiger Verdickungsmittel in Fleischwaren als Screening-Methode, Chem. Mikrobiol. Technol. Lebensm. **8**, 129-132

B 3.8 Mustranta, A. & Östman, C. (1997) Enzymatic Determination of Lactose and Galactose in Foods: NMKL Collaborative Methods Performance Study, J.AOAC Intern. **80,** 584-590

B 3.9 Saalfeld, U. & Freund, W. (1999) Charakterisierung pulverisierter Sauerteige und Möglichkeiten ihrer qualitativen Bestimmung im Brot - Teil II: Untersuchung der Acetat-, Lactose-, Hexanal-, Calcium-, Kalium- und Natriumgehalte und der Thiobarbitursäure-reaktiven Substanzen, Deutsche Lebensmittel-Rundschau **95,** 297-304

B 4.1 Henniger, G. & Hoch, H. (1981) Enzymatische Substrat-Bestimmungen in der pharmazeutischen Analytik, dargestellt an den Bestimmungen von L-Ascorbinsäure, Ethanol und Lactose, Deutsche Apotheker Zeitung **121**, 643-649

B 4.2 Saidon Sissoko (1983) Der Lactose-Gehalt in Harn und Milch des Rindes bei subklinischer B-Streptokokken-Mastitis. Dissertation. Tierärztliche Hochschule Hannover

For further information see instructions for

Test-Combination Lactose/D-Galactose	**Cat. No. 10 176 303 035**
Test-Combination D-Glucose	**Cat. No. 10 716 251 035**
Test-Combination D-Glucose/D-Fructose	**Cat. No. 10 139 106 035**
Test-Combination Sucrose/D-Glucose	**Cat. No. 10 139 041 035**
Test-Combination Sucrose/D-Glucose/D-Fructose	**Cat. No. 10 716 260 035**

R-BIOPHARM AG
An der neuen Bergstraße 17
D-64297 Darmstadt
Phone + 49 61 51 / 81 02-0
Fax + 49 61 51 / 81 02-20
www.r-biopharm.com

Roche