UV assay for the determination of lactose/D-glucose in foodstuffs and other sample materials
Test combination for 50 determinations

For *in vitro* use only
Store between 2 - 8 °C

Note: Low-lactose and lactose-free milk and milk products are usually produced using β-galactosidase. In this process, large amounts of D-glucose and D-galactose are formed from the lactose, which makes it difficult to measure the remaining, small amounts of lactose. For this reason, in compliance with § 64 LFGB 01.00-90, D-glucose is first reduced by glucose oxidase so that D-glucose released from the remaining lactose can then be measured precisely.

The Enzytec™ *Liquid* Lactose/D-Glucose test (E8130) in combination with the Enzytec™ *Liquid* D-Glucose test (E8140) and the Enzytec™ Glucose Remover (E3400) is particularly suitable for confirming the lactose-free status of dairy products.

To detect lactose concentrations in the range of the limit value of 0.01 % (10 mg/100 g), the sensitive assay performance (see chapter 4.2.) must be used.

1. Test principle

Enzymatic test with β-galactosidase (β-Gal), hexokinase (HK) and glucose-6-phosphate-dehydrogenase (G6P-DH). The reaction takes place in three steps.

Lactose is cleaved with H_2O through the presence of β-galactosidase (β-Gal) to D-glucose + D-galactose:

Lactose + H_2O $\xrightarrow{\beta\text{-Gal}}$ D-glucose + D-galactose

The resulting D-glucose is phosphorylated by a hexokinase (HK) and ATP to D-glucose-6-phosphate (G6P), which is oxidized by a glucose-6-phosphate-dehydrogenase (G6P-DH) to D-glucono-6-phosphate + nicotinamide-adenine-dinucleotide (NADH) + H^+.

D-Glucose + ATP $\xrightarrow{HK}$ D-glucose-6-phosphate + ADP

G6P + NAD^+ $\xrightarrow{G6P\text{-}DH}$ D-gluconat-6-P + NADH + H^+

NAD is reduced to NADH. The amount of NADH formed is proportional to the converted amount of lactose and free D-glucose and is measured at 340 nm.

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 50 determinations. The number of determinations for automated processing is increased by a multiple; however it depends on the device.

- Reagent 1: 2 x 50 mL with buffer, NAD, β-Gal, ATP
- Reagent 2: 2 x 12.5 mL with buffer, HK, G6P-DH

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Clarify samples containing proteins or fat with Carrez clarification.
- Filter or centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- Crush and homogenize solid or semi-solid samples and extract with water; filtrate or centrifuge, or use Carrez clarification if necessary.
- Weigh samples with a high fat content into a volumetric flask and extract with hot water; allow sample solution to cool down for fat separation (e.g. 15 min in an ice bath); fill volumetric flask up to the mark with water, filter aqueous solution before testing.
- Sample preparation in combination with the Enzytec™ Glucose Remover (E3400) is explained in more detail in the corresponding instruction for use.

4. Assays performance

4.1. General assay performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 45 - 3000 mg/L

	Reagent blank	Sample / control
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 20 min at 20 - 37 °C. Read absorbance A_1, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for 15 min at 20 - 37 °C and read absorbance A_2.		

The reagent blank value must be determined once for each run and subtracted from each sample result.

4.2. Sensitive assay performance

Sample preparation, deviating result calculation and further performance data are described in detail in a separately available application note “Determination of lactose in low-lactose and lactose-free milk and milk products“ (see chapter 7).

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of lactose

The result of the E8130 test additionally includes the amounts of free D-glucose that might be present in the sample. The sum lactose/ D-glucose is calculated with the molecular weight of lactose (342.3 g/mol) and is called *total lactose*.

$$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$$

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume} + R1}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume is increased, the test system may be affected. In general, this must be checked depending on the matrix.

$$C_{\text{total lactose}}\ [\text{g/L}] = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = 1.413 \times \Delta A$$

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 342.30
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol x cm]	= 6.3 (at 340 nm)

5.1.2. Calculation of the total lactose concentration

For the differentiation of lactose and D-glucose the free D-glucose must be determined with the Enzytec™ *Liquid* D-Glucose test (E8140). The result is subtracted from the *total lactose*:

$$C_{\text{lactose}}\ [\text{g/L}] = C_{\text{total lactose (E8130)}} - 1.9 \times C_{\text{D-glucose (E8140)}}$$

Example: Enzytec™ *Liquid* Multi-Sugar Standard low (E8440)

Total lactose (E8130)			= 1.45 g/L
D-Glucose (E8140)			= 0.50 g/L
Lactose	=	1.45 g/L - 1.9 × 0.50 g/L	= **0.50 g/L**

If the D-glucose/lactose ratio is higher than 10:1, the precision of the lactose determination decreases. In this case, the glucose excess must be eliminated with the Enzytec™ Glucose Remover kit (E3400).

5.2. Calculation of solid samples

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

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. For this purpose, we recommend the use of Enzytec™ *Liquid* Multi-Sugar Standard low (E8440).
The recovery of Enzytec™ *Liquid* Multi-Sugar Standard low and other aqueous control solutions should be within 100 ± 5 %.

6. Performance data

6.1. Specificity

The test is specific for lactose and D-glucose.

6.2. Interferences & side activities

The test shows no interferences to different relevant alcohols, acids, sweeteners and most of the sugars. In case of sulfite, there is no interference at or below 0.1 g/L. Oxalic acid and glucosamine did not interfere at or below 1 g/L.
Known side activities: allolactose is 100 % co-determined.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 3000 mg/L total lactose (sample volume of 100 µL).
The limit of detection (LoD) was determined for a sample volume of v = 100 µL according to method DIN 32645:2008-11, using buffered aqueous solutions. This results in an LoD of 10 mg/L.
The limit of quantification (LoQ) was determined by precision profile and is 45 mg/L.
The smallest absorbance difference that the method can distinguish is ΔA = 0.005. For a sample volume of v = 1000 µL, this results in an LoD of 0.95 mg/L. Based on ΔA = 0.010, an LoQ of 1.9 mg/L was calculated.

6.4. Automation with Pictus 500

6.4.1. Limit of quantification (LoQ)

P500 application	LoQ
High Range	30 mg/L
Basic Range	10 mg/L
Sensitive Range	1.25 mg/L

6.4.2. Measuring ranges

P500 application	Measuring range
High Range	to 15.000 g/L
Basic Range	to 3100 mg/L
Sensitive Range	to 310 mg/L

6.4.3. Precision and accuracy

Data from the measurement of an aqueous solution are shown here.

High Range

Target concentration, mg/L	1450	2000
Mean value, mg/L	1486	2007
SD, mg/L	26.74	22.61
RSD, %	1.80	1.13
Recovery, %	103	100

Basic Range

Target concentration, mg/L	1450	2000
Mean value, mg/L	1478	2028
SD, mg/L	5.11	8.39
RSD, %	0.35	0.41
Recovery, %	102	101

Sensitive Range

Target concentration, mg/L	145	200
Mean value, mg/L	148.0	200.9
SD, mg/L	1.29	1.22
RSD, %	0.87	0.61
Recovery, %	102	100

7. Supporting documents

On request, we offer the following documents:

- Application note for sensitive assay performance (Determination of lactose in low-lactose and lactose-free milk and milk products)
- Enzytec™ *Liquid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

9. Disclaimer

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