

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

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

Note: The present Enzytec™ Liquid Lactose/D-Galactose test (E8110) is suitable for the use in combination with the Enzytec™ Liquid D-Galactose test (E8120) for the quantitative determination of lactose in dairy products and other foods. It is also suitable for the determination of traces of lactose in non-dairy products to confirm lactose-free status.

Excluded are lactose-free milk products that have been enzymatically treated with β-galactosidase; these should be tested with the Enzytec™ Liquid Lactose/D-Glucose test (E8130) in combination with the Enzytec™ Glucose Remover (E3400).

1. Test principle

Enzymatic test with β-galactosidase (β-Gal) and galactose-dehydrogenase (Gal-DH).

Lactose is cleaved with H₂O through the presence of the enzyme β-galactosidase (β-Gal) to D-glucose + D-galactose:



The resulting D-galactose is oxidized in the presence of nicotinamide-adenine-dinucleotide (NAD) and galactose-dehydrogenase (Gal-DH) to D-galactonic acid + NADH + H⁺:



NAD is reduced to NADH. The amount of NADH formed is proportional to the amount of lactose and free D-galactose formed 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
- Reagent 2: 2 x 12.5 mL with buffer, Gal-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).
- Filter or centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- Clarify samples containing proteins or fat with Carrez clarification.

- 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.

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 30 - 2500 mg/L

	Reagent blank	Sample / control
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 40 min at 20 - 30 °C or 20 min at 37 °C. Read absorbance A ₁ , then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for 20 min at 20 - 37 °C and read absorbance A ₂ .		
Note: When performing this enzymatic test, please make sure that the incubation temperature does not fall below 20 °C, because this will lead to reduced enzyme activity and reduced recoveries.		

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

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of lactose

The result of the E8110 test additionally includes the amounts of free D-galactose that might be present in the sample. The sum lactose/D-galactose is calculated with the molecular weight of lactose (342.3 g/mol) and is called *total lactose*.

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

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.

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

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-galactose, the free D-galactose must be determined using the Enzytec™ Liquid D-Galactose test (E8120). The result is subtracted from the *total lactose*:

$$C_{\text{lactose}} [\text{g/L}] = C_{\text{total lactose}} (\text{E8110}) - 1.9 \times C_{\text{D-galactose}} (\text{E8120})$$

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

Total lactose (E8110)	=	1.45 g/L
D-Galactose (E8120)	=	0.50 g/L
Lactose =	$1.45 \text{ g/L} - 1.9 \times 0.50 \text{ g/L}$	= 0.50 g/L

If the D-galactose/lactose ratio is higher than 10:1, the precision of the lactose determination decreases.

5.2. Calculation of solid samples

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

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 $100 \pm 5 \%$.

6. Performance data

6.1. Specificity

The test is specific for lactose and free D-galactose.

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 0.5 g/L. Ascorbic acid does not interfere at or below 0.1 g/L.

Known side activities: L-arabinose and allolactose are co-determined to 100 % and lactulose to approx. 30 %.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 2500 mg/L lactose (sample volume of 100 µL).

The limit of detection (LoD) was determined for a sample volume of $v = 100 \mu\text{L}$ according to method DIN 32645:2008-11, using buffered aqueous solution. This results in an LoD 5.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile and is 30 mg/L for lactose.

The smallest absorbance difference that the method can distinguish is $\Delta A = 0.005$. For a sample volume of $v = 1000 \mu\text{L}$, this results in an LoD of 0.95 mg/L. Based on $\Delta A = 0.010$, an LoQ of 1.9 mg/L was calculated.

7. Supporting documents

On request, we offer the following documents:

- 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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