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 (36 – 46 °F)

This test was evaluated using selected samples of the following matrices: milk, milk products, infant formula, chocolate, sausage meat, almond milk, salad dressing, bread, and cookies.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test has been approved as AOAC *Official Method of Analysis* 2024.10 First Action. A publication is available in J. AOAC Int. 108(6), 901–925 (2025).

The test may be used with other foods or sample materials, provided that these are subjected to individual validation by the user.

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 (lactase); these should be tested with the Enzytec™ *Liquid* Lactose/D-Glucose test (E8130) in combination with the Enzytec™ *Liquid* D-Glucose test (E8140) and Enzytec™ Glucose Remover (E3400).

1. Test principle

Lactose is hydrolyzed to D-glucose and D-galactose in the presence of the enzyme β-galactosidase (β-Gal) and water:

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

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

D-Galactose + NAD^+ $\xrightarrow{\text{Gal-DH}}$ D-galactonic acid + NADH + H^+

NAD is reduced to NADH in this process. The amount of NADH formed during the reaction is measured at 340 nm and is equivalent to the amount of lactose and free galactose.

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 and NAD, β-Gal
- Reagent 2: 2 x 12.5 mL with buffer and Gal-DH

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 – 25 °C / 68 – 77 °F) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

3.1. General

- Sample preparation for manual and automated testing is the same.
- Samples solutions should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or dilute sufficiently to yield a concentration within the stated measuring range (refer to performance data).
- Neutralize **strongly** acidic samples by adding KOH/NaOH, or alkaline samples by adding HCl, to a pH of approx. 7.
- For turbid test samples: Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained.
- Degas samples containing carbon dioxide by, for example, stirring them in a beaker or applying a brief ultrasonic pulse (10 s).
- If necessary, decolorize **strongly** colored samples with polyvinyl-polypyrrolidone (PVPP, e.g., 1 g/100 mL sample). Stir or shake for 1 minute and filter or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- Crush and homogenize solid and semi-solid samples. Weigh a sufficient quantity of sample in a volumetric flask (considering the measuring range), extract with water; fill up to the mark and filter if necessary (by using fluted paper or syringe filters) or centrifuge in reaction tubes. Use Carrez clarification if necessary.
- For fat containing samples, weigh sufficient quantity (considering the measuring range) into a volumetric flask and extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 minutes and filter.
- Clarify samples containing proteins or fat alternatively with Carrez reagents: Weigh an appropriate sample quantity accurately into a 100 mL volumetric flask and add approx. 60 mL distilled water. In case of liquid samples, pipette the sample into a 100 mL volumetric flask or beaker pre-filled with 60 mL distilled water. Add 5 mL Carrez I solution (3.60 g potassium hexacyano-ferrate(II)-trihydrate $K_4[Fe(CN)_6]$ x 3 H_2O/100 mL) and 5 mL Carrez II solution (7.20 g zinc sulfate $ZnSO_4$ x 7 H_2O/100 mL). Mix well after each addition. Adjust the pH with 0.1 M NaOH to a value between 7.5 and 8.5. Transfer into a 100 mL volumetric flask, fill up to the mark, mix and filter using fluted paper filters or syringe filters.
- Samples with a high protein content should be clarified using concentrated Carrez reagents (see also sections 3.2.1. – 3.2.4.):
  - Carrez I solution: 15 g $K_4[Fe(CN)_6]$ x 3 H_2O/100 mL
  - Carrez II solution: 30 g $ZnSO_4$ x 7 H_2O/100 mL
- In case of higher sample volumes (up to 1000 µL), check the pH value of the test solution and neutralize in case of any doubt.

3.2. Representative Application Examples consistent with published AOAC methods

Note: The recommendations for sample preparation described below are in accordance with the published AOAC method. Alternatives may be used if properly validated and verified by the user.

3.2.1. Meat products

- Accurately weigh approx. 10 g of the homogenized sample into a resealable 50 mL plastic tube and add 20 mL distilled water.
- Vortex vigorously and fill up to 50 mL with distilled water.
- Heat for 15 minutes at 70 °C (158 °F) in a water bath.
- Add one drop of 98 % sulfuric acid and transfer the suspension quantitatively with water into a 100 mL volumetric flask.

- Allow the solution cool down to room temperature and fill the flask with distilled water up to 100 mL (fat phase above the calibration mark).
- Gently mix by inverting the flask, then filter using a paper or syringe filter.
- Use the filtrate directly for the assay or after dilution.

Important note: If *raw* meat products are analyzed, creep reactions can occur due to interfering effects of enzymes and substrates present in the raw laboratory sample. To prevent these creep reactions, the samples should be heated to approx. 75 °C (167 °F) for 15 minutes before homogenization.

3.2.2. Bakery products

- Accurately weigh approx. 2.5 g of the homogenized sample into a resealable 50 mL plastic tube and add 20 mL distilled water.
- Vortex vigorously and fill up to 50 mL with distilled water.
- Heat for 30 minutes at 50 °C (122 °F) in a water bath under occasional shaking.
- Allow to cool down to approx. room temperature and then quantitatively transfer the suspension with water into a 100 mL volumetric flask.
- Add 1 mL concentrated Carrez solution I, shake, then add 1 mL concentrated Carrez solution II and shake again.
- Fill up to 100 mL with distilled water and mix gently by inverting the flask, then filter using a paper or syringe filter.
- Use the filtrate directly for the assay or after dilution.

Note: If low very lactose and D-galactose concentrations are expected ($\Delta A \leq 0.05$), the preparation of a Carrez reagent blank (CRB) is recommended. During Carrez clarification, use 2.5 mL of distilled water instead of 2.5 g of the test sample. It is mandatory to adjust the pH with 0.1 M NaOH; otherwise, the CRB will is cloudy and the measurement will be impaired. The CRB is used instead of the reagent blank (RB) described in Section *4. Manual test procedure*.

3.2.3. Milk and milk products

- Accurately weigh approx. 1.0 g of the homogenized sample into a resealable 50 mL plastic tube, add 20 mL distilled water and vortex vigorously.
- Add 1 mL concentrated Carrez solution I, shake, then add 1 mL concentrated Carrez solution II and shake again.
- Neutralize with 0.1 M NaOH and transfer the suspension with distilled water into a 100 mL volumetric flask.
- Fill up to the mark, wait for 15 minutes, then filter using a paper or syringe filter.
- Use the filtrate directly for the assay or after dilution.

3.2.4. Cheese and chocolate

- Accurately weigh approx. 1.0 g of the homogenized sample into a resealable 50 mL plastic tube and add 30 mL distilled water.
- Heat for approx. 15 minutes at 70 °C (158 °F) in a water bath and shake the tube several times during extraction.
- Allow to cool down to room temperature.
- Add 2.5 mL concentrated Carrez solution I, shake, then add 2.5 mL concentrated Carrez solution II and shake again.
- Add 5 mL 0.1 M NaOH, shake and transfer with distilled water into a 100 mL volumetric flask.
- Fill up to 100 mL and mix; centrifuge at 4 °C (40 °F) for 10 minutes at 4000 rpm; filter using a paper or syringe filter, if necessary.
- Use the filtrate directly for the assay or after dilution.

4. Manual test procedure

Wavelength:	340 nm
Temperature (measurement):	20 – 37 °C (68 – 99 °F)
Photometer alignment:	against air (without cuvette)
Measuring range:	30 – 2500 mg/L

	Reagent blank	**Samples / controls**
Reagent 1 **Sample / control** **Dist. water**	2000 µL - 100 µL	2000 µL 100 µL -
Mix, incubate for **40 minutes at 20 – 30 °C (68 – 86 °F)** or **20 minutes at 37 °C (99 °F)**. **Read absorbance A_1**, then add:		
Reagent 2	500 µL	500 µL
Mix, incubate for **20 minutes at 20 – 37 °C (68 – 99 °F)** 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 thus reduced recoveries.		

4.1. Important notes for assay procedure

- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- Specified incubation times were verified and established at 25 °C (77 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- Use separate tips for each sample and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance
- Always wait for the reaction to end (at least during the first test runs or validation). If the absorbance has not stabilized after the recommended incubation time, continue measuring at 5-minute intervals, for example, until a constant absorbance value is reached.
- If a creep reaction occurs, the reaction will not have finished after stated incubation times and will typically show a constant increase of ΔA. Calculate the analyte-specific ΔA value by plotting the absorbance values against time and performing a linear regression to determine the rate of increase in ΔA per minute related to the creep reaction. Then, extrapolate the absorbance to the time at which reagent 2 is added.
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary.

5. Calculation of results

5.1. Calculation of the concentration in sample solutions

5.1.1. Calculation of the total lactose concentration (sum of lactose and free D-galactose)

The results of this test also include the amounts of free D-galactose that may be present in the sample. The sum of lactose and D-galactose is calculated using the molecular weight of lactose (342.3 g/mol) and is referred to as total lactose.

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

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

The specified df value of **0.808 applies to a base application of 100 µL**. An increase in sample volume is possible (max. 1000 µL; refer to validation report). **While keeping reagent volumes unchanged**, this requires **conversion of the reagent dilution factor (df)** accordingly.

Increasing the sample volume may influence test performance. This must generally be checked depending on the matrix. **The reagent blank value must be adjusted to the changed sample volume.**

The concentration of *total lactose* is calculated using Lambert-Beer's law:

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

If the sample solution was diluted before measurement, this result has to be multiplied with the **sample pre-dilution factor F**.

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

5.1.2. *Calculation of the real lactose concentration*

To differentiate between lactose and D-galactose, free D-galactose must be determined separately using the Enzytec™ *Liquid* D-Galactose E8120 test. The result is subtracted from the total lactose. This requires taking into account the ratio of the molecular weights of the two types of sugar:

MW_{lactose} 342.3 g/mol : $MW_{\text{D-galactose}}$ 180.16 g/mol → factor 1.9

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

Additional information on how to use Enzytec™ *Liquid* D-Galactose E8120 can be found in the accompanying package insert.

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

Total lactose (E8110)		= 1.45 g/L
D-Galactose (E8120)		= 0.50 g/L
Real lactose =	1.45 g/l - 1.9 × 0.50 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 the content in solid samples*

When analyzing solid and semi-solid samples that have to be weighed in for the extraction of the sample, the content is related to the sample weight:

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

5.3. *Controls & acceptance criteria*

Control or reference samples should be included in each run for quality control purposes. Therefore, we recommend Enzytec™ *Liquid* Multi-Sugar Standard *low* (Art. No. E8440; with 0.50 g/L lactose and 0.50 g/L D-galactose).

The theoretical target value for total lactose is calculated as follows:

Total lactose = (0.50 g/L × 1.9) $_{\text{D-galactose}}$ + 0.50 g/L $_{\text{lactose}}$ = 1.45 g/L

The recovery of this multi-standard *low* and other *aqueous* control solutions should be 100 ± 5 %.

As certified reference materials, we recommend, among others:

- NIST 1849a *Infant formula,* lactose monohydrate
- LGC 7016 *Chocolate confectionary,* lactose anhydrous
- QSE Reference *Raw Milk*, F3 # D00M09Y16

The recovery of QSE materials and *extracted* matrices should be within 100 ± 10 %.

6. Performance data

6.1. *Specificity & side activities*

The test is specific for lactose and free D-galactose. The shows a side activity towards L-arabinose, where L-arabinose is oxidized by the galactose-dehydrogenase for about 120 %.

Allolactose (6-O-(β-D-galactopyranosyl)-D-glucopyranose), 3-O-(β-D-galactopyranosyl)-D-glucopyranose and 3-galactosyllactose showed activities around 100 %.

6.2. *Interferences*

The following substances were investigated at concentrations ranging from 1 g/L to 10 g/L in presence of 0.25 g/L lactose and 0.25 g/L D-galactose (in sum 0.725 g/L total lactose): galactaric acid, ascorbic acid, D-/L-lactic acid, acetic acid, D-/L-tartaric acid, citric acid, gluconic acid oxalic acid, benzoic acid and D-/L-malic acid. Except for ascorbic acid, none of the substances showed a significant interference. Ascorbic acid does not interfere at concentrations of 1g/L or lower in the lactose/D-galactose system.

Additionally, the following substances were tested for interference: sodium chloride (NaCl), taurine, glucosamine, ethanol, and sulfite. None of them interfered at concentrations of 10 g/L or more except for sulfite, which exerted an interference at 0.5 g/L of sulfite. The recoveries were acceptable at 0.05 g/L.

Several sugar substitutes were tested at a concentration of 10 g/L in in presence of 0.25 g/L lactose and 0.25 g/L D-galactose (in sum 0.725 g/L total lactose) during validation for interference: sorbitol, mannitol, isomalt, maltitol, lactitol, xylitol, erythritol, inulin, isomaltulose, fructose, corn syrup, oligofructose and trehalose. With the exception of lactitol, none of the substances showed an interfering effect at 10 g/L in the lactose/D-galactose system. However, lactitol does not interfere at concentrations of 1 g/L or less.

Various sweeteners were tested at 1 g/L, 2 g/L or 5 g/L for their interference in the presence of 0.25 g/L lactose and 0.25 g/L galactose: acesulfame, adventame, aspartame, cyclamate, neohesperidine, neotame, saccharin, sucralose, thaumatin, and alitame showed no interfering effect in the determination of total lactose.

6.3. *Linearity, measuring range & sensitivity*

Linearity is given up to 2500 mg/L D-galactose (sample volume of 100 µL) with a recommended measuring range of 30 – 2500 mg/L.

The limit of detection (LoD) was determined according to the DIN 32645:2008-11 method in a buffered aqueous solution. For a sample volume of 100 µL, the calculated LoD is 5.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 30.0 mg/L for a sample volume of 100 µL.

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.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Lactose/D-Galactose Validation Report
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Lactose/D-Galactose Excel template for results
- Enzytec™ *Liquid* Lactose/D-Galactose Technical information
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:

https://eifu.r-biopharm.com/

8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

Upon request, we offer the following services, among others:

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

10. Disclaimer

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