UV assay for the determination of 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 samples, whey and skimmed milk powder, infant formula, cheese, yogurt, ice cream, chocolate, sausage and soy based products.

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

This test Enzytec™ *Liquid* D-Galactose E8120 was approved as AOAC *Official Method of Analysis* 2024.10 in conjunction with Enzytec™ *Liquid* Lactose/D-Galactose E8115. A publication is available in J. AOAC Int. 108(6), 901–925 (2025).

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

1. Test principle

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

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

NAD is reduced to NADH. The amount of NADH formed is stoichiometric to the amount of (free) D-galactose in the sample and is measured at a wavelength of 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 and NAD
- 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

- Sample preparation for manual and automated testing is the same.
- Samples solutions should be brought to room temperature before measurement.
- Use liquid, clear, colorless and almost neutral sample solutions directly or dilute sufficiently to yield a D-galactose concentration within the stated measuring range (refer to performance data).
- 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.
- 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 aid of a short ultrasound burst (e.g., 10 seconds); filter, if solution is not clear.
- Neutralize strongly acidic samples by adding KOH/NaOH, or alkaline samples by adding HCl, to a pH of approx. 7.
- Decolorize strongly colored samples with polyvinylpolypyrrolidone (PVPP), e.g. by adding 0.1 g PVPP to 10 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 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.

Please note: Specific sample preparations for foods such as meat products, baked goods, milk and dairy products, as well as cheese and chocolate, can be found in the test kit instructions for Enzytec™ *Liquid* Lactose/D-Galactose (Art.No. E8110) or Enzytec™ *Liquid* Combi Lactose/D-Galactose (Art.No. E8115).

4. Manual test procedure

Wavelength: 340 nm
Temperature: 20 – 37 °C (68 – 99 °F)
Photometer alignment: against air (without cuvette)
Measuring range: 8 – 2000 mg/L (for 100 µL sample)

	Reagent blank	**Samples / controls**
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for **3 minutes at 20 – 37 °C (68 – 99 °F)**. **Read absorbance A_1**, then add:		
Reagent 2	500 µL	500 µL
Mix, incubate for **15 minutes at 20 – 37 °C (68 – 99 °F)** and **read absorbance A_2**.		

4.1. Important notes for assay procedure

- The reagent blank value (water sample) **must be determined in each series of measurements** and subtracted from **each** sample result.
- Specified incubation times were validated 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 extract 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 or for the absorbance to stabilize (at least during the first test runs or validation). If the absorbance has not stopped 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 D-galactose concentration

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

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

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 D-galactose is calculated using Lambert-Beer's law:

$$\mathbf{C_{D\text{-}galactose}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.744 \times \Delta A} \times F$$

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 D-galactose [g/mol]	= 180.16
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol × cm]	= 6.3 (at 340 nm)

5.2. Calculation of the D-galactose 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:

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

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; 0.50 g/L D-galactose).

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

For the preparation of control or standard solutions, we recommend using the following materials, for example:

- D-Galactose ≥ 99 %, anhydrous (Carl Roth, Art. No. 8878.2); c = 1 g/L galactose in dist. water; stable for approx. one week at 4 – 8 °C (39 – 46 °F).

6. Performance data

6.1. Specificity & side activities

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

6-O-(β-D-galactopyranosyl)-D-galactopyranose also exhibited a side activity, with a recovery rate of approximately 50 %.

6.2. Interferences

The following substances were investigated at concentrations ranging from 1 g/L to 58 g/L in the multi-sugar standard: ascorbic acid, SO_2, D-/L-lactic acid, acetic acid, D-/L-tartaric acid, citric acid, D-/L-malic acid, NaCl, taurine, glycerine, oxalic acid, benzoic acid, gluconic acid, mucic acid, glucosamine, β-lactoglobulin and casein. No interference was observed for any of the tested substances. Sulfite does not interfere at or below ≤ 0.5 g/L, whereas ascorbic acid does not interfere at or below ≤ 10 g/L.

Several sugar substitutes were tested at a concentration of 10 g/L in a multi-sugar standard (containing 0.25 g/L of galactose) during validation for interference: sorbitol, mannitol, isomalt, maltitol, lactitol, xylitol, erythritol, inulin, isomaltulose, fructose, corn syrup, oligofructose and trehalose showed no interfering effect in the determination of D-galactose.

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 D-galactose: acesulfame, adventame, aspartame, cyclamate, neohesperidine, neotame, saccharin, sucralose, thaumatin, and alitame showed no interfering effect in the determination of D-galactose.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 2000 mg/L D-galactose (sample volume of 100 µL) with a recommended measuring range of 8 – 2000 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 2.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 8.0 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 0.5 mg/L. Based on ΔA = 0.010, an LoQ of 1.0 mg/L was calculated.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* D-Galactose Validation Report
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* D-Galactose Excel template for results
- Enzytec™ *Liquid* 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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