

Enzymatic assay for Lactose/D-Galactose in foodstuff and other sample materials
2 x 50 ml R1 / 2 x 12.5 ml R2 (50 assays)

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

Principle

Enzymatic test with β-Galactosidase (β-Gal) and Galactose Dehydrogenase (Gal-DH). NADH is produced and is measured at 340 nm:
Lactose + H₂O — β-Gal —> D-Glucose + D-Galactose
D-Galactose + ATP — Gal-DH —> D-Galactonic acid + NADH

Reagents

The reagents are ready-to-use.

Reagent 1: two vials ≥ 50 ml (NAD, β-Gal)

Reagent 2: two vials ≥ 12.5 ml (Gal-DH)

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C. Do not freeze the reagents. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

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 material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

- Use liquid and clear samples directly, or after dilution into the relevant measuring range (see test performance)
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- 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
- For fat containing samples, weigh sample into a volumetric flask (min. 50 ml) and extract with hot water; cool to allow the fat to separate; make up to the mark with water, remove the fatty layer on the top and filter the aqueous part

Assay procedure

Wavelength: 340 nm
Optical path: 1 cm
Temperature: 20 – 25 °C
Measurement: Against air or against water
Sample solution: 30 – 2000 mg/l

	Reagent Blank (RB)	Samples
Sample / Standard	-	100 µl
Dist. water	100 µl	-
Reagent 1	2000 µl	2000 µl
Mix, incubate for approx. 20 min. at 20 – 25 °C. Read absorbance A1, then add:		
Reagent 2	500 µl	500 µl
Mix, wait until the end of the reaction (≥ 15 min. at 25 °C or ≥ 40 min at 20°C). Read absorbance A2.		

Reagent blank must be performed once for every run, and subtracted from every sample during calculation of results.

Calculation of results

Total-Lactose (Lactose + free D-Galactose)

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

With df = dilution factor of optical densities, because of reagent volumes:

$$df = (\text{sample} + R1) / (\text{sample} + R1 + R2) = 0.808.$$

$$c = (V \times MW \times \Delta A) / (\epsilon \times d \times v \times 1000) \text{ [in g/l of Total-Lactose]}$$

$$c = (2.600 \times 342.30 \times \Delta A) / (\epsilon \times 1 \times 0.1 \times 1000)$$

It results for the determination at 340 nm ($\epsilon = 6.3 \text{ l} \times \text{mmol}^{-1} \times \text{cm}^{-1}$):

$$C_{\text{Total Lactose}} [\text{g/l}] = 1.413 \times \Delta A$$

Calculation of Real Lactose

The result above includes the amount of Lactose plus the free D-Glucose which is present in the sample. It is calculated as "Total Lactose", with the molecular weight of Lactose (342.3 g/mol). For differentiation of the two sugars, the free D-Galactose must be measured with the Enzytec™ Liquid D-Galactose assay (E8120) in a separate run. Lactose is calculated by subtraction of the free D-Galactose from the Total Lactose, taking into account the ratio between the molecular weights of both sugars:

$$C_{\text{Lactose}} [\text{g/l}] = C_{\text{Total-Lactose}} - 1.90 \times C_{\text{Galactose}}$$

Example:

Total-Lactose (E8110) 1.500 g/l
D-Galactose (E8120) 0.400 g/l
Lactose = 1.500 g/l – 1.90 x 0.400 g/l = 0.740 g/l

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

Test performance

Specificity

The test is specific for Lactose and free D-Galactose. Apart from D-Galactose, Gal-DH also oxidizes L-Arabinose to 100%. Allolactose und Lactulose are recognized by β-Gal with approx. 80% resp. 30%.

Measuring range

The recommended measuring range is 50 - 2000 mg/l (Lactose and D-Galactose). When values exceed this range, samples should be diluted into the range 100 to 2000 mg/l.

Sensitivity

The Limit of Detection (LoD) and Limit of Quantification (LoQ) where determined according to the method DIN 32645:2008-11:

- LoD = 30 mg/l (Total Lactose)
- LoQ = 50 mg/l (Total Lactose)

Automation

Application sheets for automated systems are available on request.

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