Enzymatic assay for 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 Galactose Dehydrogenase (Gal-DH). NADH is produced and is measured at 340 nm:
D-Galactose + ATP —— Gal-DH ⟶ D-Galactonic acid + NADH

Reagents

The reagents are ready-to-use.

Reagent 1: two vials ≥ 50 ml (NAD)

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: 15 – 1000 mg/l

	Reagent Blank (RB)	Samples
Sample / Standard	-	100 µl
Dist. water	100 µl	-
Reagent 1	2000 µl	2000 µl
Mix, incubate for approx. 3 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

Sample solution

$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$
df = dilution factor of optical densities because of reagent volumes
df = (sample volume + R1) / (sample volume + R1 + R2) = 0.808.

c = (V x MW x Δ A) / (ε x d x v x 1000) [in g/l of D-Galactose] with:

V	(Total volume)	= 2.600 [ml]
MW	(Molecular weight)	= 180.16 [g/mol]
d	(Optical path)	= 1.00 [cm]
v	(Sample volume)	= 0.100 [ml]
ε	(Extinction coefficient NADH) [l x mmol^{-1} x cm^{-1}] :	
	340 nm = 6.3 334 nm = 6.18 365 nm = 3.4	

c = (2.600 x 180.16 x Δ A) / (ε x 1 x 0.1 x 1000)
It results for a determination at 340 nm:
$c_{D\text{-}Galactose}$ [g/l] = **0.744** x ΔA

Calculation in solid samples

$$\text{Content [g/100 g]} = \frac{C_{test}\ [g/l]}{weight_{sample}\ [g/l]} \times 100$$

Test performance

Specificity

The test is specific for D-Galactose. Apart from D-Galactose, Gal-DH also oxidizes L-Arabinose to 100%.

Measuring range

The recommended measuring range is 25 - 1000 mg/l. When values exceed this range, samples should be diluted into the range 50 to 1000 mg/l.

Sensitivity

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

- LoD = 15 mg/l (D-Galactose)
- LoQ = 25 mg/l (D-Galactose)

Automation

Application sheets for automated systems are available on request.

Disclaimer

The data corresponds to our present state of technology and provides information on our products and their uses. R-Biopharm makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Defective products will be replaced. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. R-Biopharm shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.