Enzymatic determination of Lactose and D-Galactose in foodstuff and other sample materials
Test-kit for 32 determinations on the RIDA®CUBE SCAN instrument (340 nm)

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

Principle

Enzymatic test with ß-Galactosidase (ß-Gal) and Galactose Dehydrogenase (Gal-DH). NADH is produced and is measured at 340 nm:

Lactose + H_2O —— ß-Gal ⟶ D-Glucose + D-Galactose

D-Galactose + NAD^+ —— Gal-DH ⟶ D-Galactonic acid + NADH

Reagents

1: 32 tubes with 800 µl reagent 1 (NAD, ß-Gal)

2: 32 caps with 200 µl reagent 2 (Gal-DH)

3: one RFID-card (Radio Frequency Identification)

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 clear and transparent samples directly, or after dilution into the relevant measuring range
- Filter or centrifuge turbid solution
- Degas samples containing carbon dioxide
- Clarify samples containing proteins or fat with Carrez clarification
- Crush and homogenize solid samples and extract with water; use Carrez clarification if necessary, filter or centrifuge
- For the ultra-sensitive application (200 µl sample), strongly acidic or alkaline samples should be adjusted to pH approx. 7.5, by adding NaOH / HCl. This step is not necessary for the basic application (20 µl sample).

Assay specifications

The assay specifications are saved on the RFID card and are executed automatically by the instrument.

Wavelength:	340 nm
Temperature:	37 °C
Calibration:	calibration curve saved on RFID card
Test sequence:	sample + R1 / mix / 10 min / A1 / R2 / mix / 10 min / A2
Sample volume:	20 µl (basic) or 200 µl (ultra-sensitive). The required volume should be pipetted precisely into the test tube (reagent 1).

For the ultra-sensitive application, it is also possible to pipette any dilution with 200 µl total volume (for example 50 µl sample and 150 µl water). Results must be recalculated accordingly (in this example, multiply by factor 4).

Handling procedure

Place the RFID-Card on the instrument	
Enter sample data into the tablet app : - identification - volume (20 or 200 µl)	
Pipette the sample into the test-tube (reagent 1)	 
Close the tube with the cap (reagent 2), insert it into the instrument and close the door	 

Calculation of results

The results are given in mg/l by the instrument, and following ranges are recommended:
- from 100 to 4000 mg/l for the basic application (20 µl)
- from 10 to 400 mg/l for the ultra-sensitive application (200 µl)

The result includes the amount of Lactose plus the free D-Galactose 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 RIDA®CUBE D-Galactose assay (RCS4120) in a separate run. Lactose is calculated by subtraction of the free Galactose content from the Total-Lactose content, taking into account the difference between the molecular weights of both sugars (factor 1.9):

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

Example:

Total-Lactose (RCS4110)	*1500 mg/l*
D-Galactose (RCS4120)	*400 mg/l*
Lactose = 1500 mg/l – 1.90 x 400 mg/l	*= 740 mg/l*

Notes

Use a quality control every day where a run is performed. If the deviation of this quality control is higher than 10%, it is recommended to measure the reagent blank with a water sample, and to subtract it from all future sample results.