UV method for approx. 16 assays each / approx. 32 assays lactose

For laboratory use only
Store between +2 and +8°C

The method is contained in the Austrian, Dutch, German, and Swiss food laws. Recommended e.g. by IDF, VDLUFA. Standardized by DIN (Germany), GOST (Russia), NBN (Belgium). Approved by AOAC.

Principle

Lactose + H_2O ——— β-galactosidase ⟶ D-glucose + D-galactose
D-galactose + NAD^+ ——— β-Gal-DH ⟶ D-galactonic acid + NADH + H^+

Ref.: Beutler, H.O. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 3rd ed., vol. VI, pp. 104-112, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel

Assay performance

Wavelength:	340 nm (NADH) ε = 6.3 l x $mmol^{-1}$ x cm^{-1}
Light path:	+1.00 cm (glass or plastic cuvettes)
Temperature:	+20 to +25°C
Assay volume:	3.300 ml
Measurement:	against air or against water
Sample solution:	4 to 200 µg lactose + D-galactose in 0.100 to 0.500 ml sample solution

Reagents

1 Lyophilizate with citrate buffer, pH approx. 6.6, approx. 35 mg NAD (for stability see pack label). *Dissolve contents of bottle # 1 with 7 ml redist. water.* The solution is stable for 3 months at +2 to +8°C.

2 Approx. 1.7 ml β-galactosidase suspension (approx. 100 U) in ammonium sulfate (for stability see pack label). *The suspension is ready for use.* Swirl bottle carefully before the suspension is pipetted.

3 Approx. 34 ml potassium diphosphate buffer, pH approx. 8.6 (for stability see pack label). *The solution is ready for use.*

4 Approx. 1.7 ml β-galactose dehydrogenase (approx. 40 U) in ammonium sulfate (for stability see pack label). *The suspension is ready for use.* Swirl bottle carefully before the suspension is pipetted.

In addition (not contained in the kit):
Standard solution lactose-monohydrate, ultrapure, 1 g/l for test control only.
Standard solution D-galactose, ultrapure, 0.5 g/l for test control only.

The reagents for the determination of lactose/D-galactose are not hazardous. The general safety rules for the work in chemical laboratories should be applied. After use the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

If the sample has one of the characteristics below, which hamper the test, please follow the corresponding sample preparation procedure:

1. Dilute *clear, colourless and almost neutral liquid samples* to get a sample solution with 0.1 to 1 g lactose + D-galactose/l.
2. Filter or centrifuge *turbid solutions*, dilute (see pt. 1).
3. Degas *samples containing carbon dioxide*, e.g. by filtration, or add $NaHCO_3$ till the solution is slightly alkaline, dilute (see pt. 1).
4. Crush (corn size < 0.3 mm) or homogenize *solid or semi-solid (pasty) samples*, extract with water, or dissolve in water, filter and dilute (see pt. 1) if necessary.
5. Extract *fat containing samples* with hot water at a temperature above the melting point of fat, e.g. in a 100 ml volumetric flask. Adjust to +20°C, fill volumetric flask to the mark. Store in ice or in refrigerator for approx. 15 resp. 30 min, filter.
6. Clarify *samples containing protein* with Carrez reagents.
7. Deproteinize *samples containing protein* with perchloric acid.

Procedure for lactose / D-galactose (approx. 16 assays each)

Pipette into cuvettes	**Blank lactose sample**	**Lactose standard[1] assay**	**Lactose sample[2,3] assay**	**Blank D-galactose sample**	**D-Galactose sample assay**	**Internal standard[4]**
Citrate buffer, NAD solution # 1	0.200 ml	0.200 ml	0.200 ml	0.200 ml	0.200 ml	0.200 ml
β-Galactosidase suspension # 2	0.050 ml	0.050 ml	0.050 ml	-	-	0.050 ml
Sample sol.[6] (e.g. 0.1 to 1 g lactose/l)	**-**	**-**	**0.100 ml**	**-**	**0.100 ml**	**0.100 ml**
Standard solution[6] (e.g. 1 g lactose/l)	-	0.100 ml	-	-	-	0.100 ml
Mix e.g. by gentle swirling of the cuvette. Incubate at +20 to +25 °C for at least 30 min. Add:						
Potassium diphosphate buffer, solution # 3	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000	1.000 ml
Redist. Water	2.000 ml	1.900 ml	1.900 ml	2.050 ml	1.950 ml	1.800 ml
Mix[7], after approx. 3 min read the absorbances (A_1). Add:						
β-Gal-DH suspension # 4	0.050 ml	0.050 ml	0.050 ml	0.050 ml	0.050 ml	0.050 ml
Mix[7], after approx. 30 min read the absorbances (A_2). Repeat absorbance reading after another 5 min[8].						

See notes on next page

Procedure for lactose via D-galactose (approx. 32 assays)

If the sample does not contain free D-galactose, the D-galactose assay is omitted (see note n° 9).

Pipette into cuvettes	**Blank lactose sample[9]**	**Lactose standard[1,9] assay**	**Lactose sample [2,9] assay**	**Lactose rerun[3,9] assay**	**Internal standard[4,9]**	**Lactose high sensitive assay[5,9]**
Citrate buffer, NAD solution # 1	0.200 ml	0.200 ml	0.200 ml	0.200 ml	0.200 ml	0.200 ml
β-Galactosidase suspension # 2	0.050 ml	0.050 ml	0.050 ml	0.050 ml	0.050 ml	0.050 ml
Sample sol.[6] (e.g. 0.1 to 1 g lactose/l)	**-**	**-**	**0.100 ml**	**0.200 ml**	**0.100 ml**	**0.500 ml**
Standard solution[6] (e.g. 1 g lactose/l)	-	0.100 ml	-	-	0.100 ml	-
Mix e.g. by gentle swirling of the cuvette. Incubate at +20 to +25 °C for at least 30 min. Add:						
Potassium diphosphate buffer, solution # 3	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000	1.000 ml
Redist. Water	2.000 ml	1.900 ml	1.900 ml	1.800 ml	1.800 ml	1.500 ml
Mix[7], after approx. 3 min read the absorbances (A_1). Add:						
β-Gal-DH suspension # 4	0.050 ml	0.050 ml	0.050 ml	0.050 ml	0.050 ml	0.050 ml
Mix[7], after approx. 30 min read the absorbances (A_2). Repeat absorbance reading after another 5 min[8].						

Calculation

$\Delta A = (A_2 - A_1)_{\text{sample resp.standard}} - (A_2 - A_1)_{\text{blank}}$

$\mathbf{C}_{\text{lactose/D-galactose}}$ = (V x MW x Δ A) / (ε x d x v x 1000) [g D-galactose, resp. lactose /l sample solution]

Lactose/D-galactose assay

To obtain the true lactose value, the result from the D-galactose assay has to be subtracted:

$\Delta A_{\text{lactose}} = [(A_2 - A_1)_{\text{assay lactose}} - (A_2 - A_1)_{\text{blank lactose}}] - [(A_2 - A_1)_{\text{assay D-galactose}} - (A_2 - A_1)_{\text{blank D-galactose}}]$

c = (3.300 x 342.3 (resp. 360.32) x ΔA) / (6.3 x 1.00 x 0.100 x 1000) = **1.793 (resp. 1.887) x Δ A [g/l lactose (resp. lactose monohydrate)]**

For D-galactose: **c =** (3.300 x 180.16 x ΔA) / (6.3 x 1.00 x 0.100 x 1000) = **0.9437 x Δ A [g/l D-galactose]**

Lactose via galactose assay[9]

If the sample does not contain free D-galactose, no D-galactose assay needs to be performed. If some free D-galactose is present in the sample, it will be calculated as lactose.

$\Delta A = (A_2 - A_1)_{\text{sample resp.standard}} - (A_2 - A_1)_{\text{blank}}$

c = (V x MW x Δ A) / (ε x d x v x 1000) [g lactose /l sample solution]

c = (3.300 x 342.3 (resp. 360.32) x Δ A) / (6.3 x 1.00 x 0.100 x 1000) = **1.793 (resp. 1.887) x Δ A [g/l lactose (resp. lactose monohydrate)]**

In all cases, the calculation of results can be done on the basis of anhydrous lactose or of lactose monohydrate (as in traditional analysis).
If the sample has been diluted during preparation, multiply the result with the dilution factor F.
When analyzing samples which are weighed out for sample preparation, calculate the content from the amount weighed.

Assay characteristics

1. *Specificity* Specific for lactose under assay conditions. Relatively specific for D-galactose in the absence of L-arabinose. In the analysis of commercial lactose monohydrate and D-galactose results of < 100 % have to be expected because the materials absorb moisture.
2. *Sensitivity*: 2 mg lactose/l (Δ A=0.005; v=0.500 ml ; V=3.300 ml)
   1 mg D-galactose/l (Δ A=0.005; v=0.500 ml ; V=3.300 ml)
3. *Detection limit:* 7 mg lactose/l (Δ A=0.020; v=0.500 ml; V=3.300 ml)
   4 mg D-galactose/l (Δ A=0.020; v=0.500 ml; V=3.300 ml)
4. *Linearity:* 4 µg lactose+ D-galactose/assay (v = 0.500 ml; V = 3.300 ml)
   to 200 µg lactose + D-galactose/assay (v = 0.100 ml; V = 3.300 ml)
5. *Precision:* Δ A = 0.005 – 0.010 absorbance units (D-galactose),
   Δ A = 0.010 – 0.015 absorbance units (lactose)
   CV = approx. 1 to 2 % (lactose, D-galactose)

Notes

1 Run a "standard" to see "accidents" in analysis. The measurement of the standard is not necessary for calculating results.
2 This assay together with the blank is a single determination.
3 In the case of a double determination, run two assays with different sample volumes. The absorbance differences measured have to be proportional to the sample volumes.
4 Recovery = $[(\Delta A_{\text{sample+standard}} - \Delta_{\text{sample}}) / \Delta_{\text{standard}}] \times 100$ [%]
5 In the case of trace level compound analysis, the sample volume can be increased up to 0.500 ml (0.007 to 0.4 g lactose/l).
6 Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution.
7 e.g. with a plastic spatula, or after closing the cuvette with Parafilm (trademark of American Can Co., Greenwich Ct., USA).
8 The reaction has stopped when the absorbance is constant. If the reaction has not stopped, continue to read absorbances until the absorbances increase constantly over e.g. 5 min. Extrapolate absorbances to the time of the addition of β-Gal-DH (suspension # 4).
9 Many foodstuffs do not contain free D-galactose, therefore its determination can be omitted. If the sample does contain free D-galactose, it will be calculated as lactose.