Enzymatic method for wines, food and beverages.
4 x 20 mL R1 + 4 x 20 mL R2 + 2 x 8.5 mL R3 + 2 x 8.5 mL R4 + 1 x 5 mL R5 + 1 x 5 mL R6
(400 tests on automatic analyzer)

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

Principle

The determination of sodium by enzymatic way is carried out through the use of a β-galactosidase (GAL) with sodium-dependent activity, in the presence of ONPG (o-nitrofenil-β-D-galactopyranoside) as substrate. The increase in absorbance at 405 nm allows to evaluate the concentration of sodium in the sample.
Using the standards contained in the kit, it is possible to prepare a 2-point calibration curve. By reporting the absorbance values of the individual samples on this curve, their concentration can be determined.

Assay specifications

Wavelength: 405 nm
Path width: 1.00 cm
Measurement: against air or distilled water
Temperature: 37 °C
Method: Endpoint (2 point-end)
Reaction time: 8 minutes
Linearity: up to 4 g/L

Reagents

1: Buffer (> 0.050 mol/L): 4 bottles of approx. 20 mL

2: R2 - GAL (GAL < 50 KU/L): 4 bottles of approx. 20 mL

3: R3 - DIL (Diluent < 0.2 mol/L): 4 bottles of approx. 8.5 mL

4: R4 - ONPG (ONPG < 50 mmol/L): 2 bottles of approx. 8.5 mL

5: Liquid Standard 1: 1 vial of approx. 5 mL
(Na^+ = 2.759 g/L)

6: Liquid Standard 2: 1 vial of approx. 5 mL
(Na^+ = 4.138 mg/L)

All reagents are ready to use. Bring the reagents to working temperature before use. Stir gently before adding. Close immediately after use.

This product has been formulated for in vitro diagnostic use. The reagent should only be used for the purpose indicated by experienced and trained personnel. The reagents contain sodium azide as a preservative, in a total concentration below the limits set out in Dir.67/548/EEC and 88/379/EEC and related amendments for the classification, labelling and packaging of dangerous preparations (reagents).

Do not ingest. Avoid contact with skin and mucous membranes. On the material safety data sheet are detailed the operating procedures for the manipulation of this product. Material safety data sheet can be supplied on request.

After use, the reagents must be disposed of as laboratory waste.

Stability of reagents

Closed reagents are stable until the expiration date indicated on the label, when stored in their undamaged primary container between 2 and 8 °C, provided that they have not been contaminated during their use. If the primary container is damaged, dispose of it.

Preparation of the R2 - GAL working reagent

Add 20 mL of **R1 - BUFFER** to a vial of **R2 - GAL**. Stir gently until completely dissolved. Avoid foaming. Bring the reagents to working temperature before use. Close immediately after use.

Stability of the R2 - GAL working reagent

The working reagent is stable for 2 weeks at 2 - 8 °C.

Preparation of the R4 - ONPG starter reagent

Add 8.5 mL of **R3 - Diluent** to a vial of **R4 - ONPG.** Stir gently until completely dissolved. Avoid foaming. Bring the Reagent to working temperature before use. Close immediately after use.

Stability of the R4 - ONPG starter reagent

The R4 - ONPG starter reagent is stable for 4 weeks.

Sample preparation

- Wine can be analyzed directly.
- Use liquid, clear and nearly neutral samples directly or after dilution into the relevant measuring range (up to 4 g/L, see performance data).
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide.
- Crush and homogenize solid samples, weigh out appropriate sample amount and extract with water.

Test Procedure

Pipette into cuvettes:	Standard (ST)	Sample (S)
Work reagent **R2 - GAL**	2000 µL	2000 µL
Sample	-	40 µL
Standard	40 µL	-
Mix gently each cuvette and incubate for 5 minutes at 37 °C. Then add:		
Reagent starter **R4 - ONPG**	400 µL	400 µL
Mix gently and after 30 seconds at 37 °C read the Abs1 absorbance of the Standard (ST) and the sample (S). Wait another 2 minutes exactly and read the Abs2 absorbance of the Standard (ST), of the Sample (S).		

Note: Because of the precise timing of A1 after 30 seconds and A2 after another 2 minutes, it challenging to perform this test on a manual photometer. This test kit and application has been validated only on automated analyzers.

Calculation of results

Calculate for each point of standard:

$\Delta Abs_{ST} = (Abs1_{ST} - Abs2_{ST})$

And for each sample:

$Abs_S = (Abs1_S - Abs2_S)$

Report for each calibrator the values of ΔAbs_{ST} against the concentration of the standard to construct the calibration curve.

The calibration curve must always be repeated at each change of batch, reagent and/or calibrator.
Report each Abs_S value found on the calibration curve to determine the concentration of the analyzed samples.

Performance data

1. No interference is known.
2. Linearity of method:
   The test is linear up to 4 g/L. However, for sodium concentrations above 4 g/L, it is recommended to dilute the sample with distilled water, retest and multiply the result by the dilution factor.
3. Method sensitivity (LoD):
   The sensitivity limit, i.e. the minimum concentration that can be distinguished from zero, is 72 mgl/L.
4. A proportional change in reaction volumes does not change the result.
5. Do not mix reagents from different production batches together.
6. Applications on automatic chemistry analyzers are available upon request.

References

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3. CLSI(NCCLS) GP44-A4/H18-A4: Proc. for the Handling and Processing of Blood Specimens for Common Lab. Tests.
4. Berry M.N. et al., Clin. Chem. 342295 (1988).

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