Enzymatic method for wines, food and beverages
4 x 20 mLR1 + 4 x 20 mL R2 + 2 x 8.5 mL R3 + 1 x 5 mL R4 + 1 x 5mL R5
(400 tests on automatic analyzer)

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

## Principle

The enzymatic determination of Potassium is using a potassium-dependent Pyruvate Kinase ($K^+$-PK) and Lactate Dehydrogenase (L-LDH):

Phosphoenolpyruvate + ADP — $K^+$-PK ⟶ Pyruvate + ATP
Pyruvate + NADH — L-LDH ⟶ Lactate + NAD

The decreasing amount of NAD is measured at 340 nm and is proportional to the $K^+$ concentration in the sample.

## Assay specifications

Wavelength: 340 nm (334 - 365 nm)
Path width: 1.00 cm (glass; plastic)
Temperature: 37 °C
Method: endpoint
Reaction time: 5 minutes
Measurement: against air or distilled water
Linearity: up to 320 mg/L

## Reagents

# 1: Buffer (> 0.050 mol/L): 4 x approx. 20 mL

# 2: R2 - PK (PK > 50 KU/L): 4 vials give approx. 20 mL each

# 3: R3 - LDH ready to use (< 300 KU/L): 2 x approx. 8.5 mL

# 4: Liquid Standard 1: approx. 5 mL ($K^+$ = 97.75 mg/L)

# 5: Liquid Standard 2: approx. 5 mL ($K^+$ = 293.25 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 can be supplied on request.

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

Stability:
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.

## Preparation of the working reagent

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

Close immediately after use. The products must be handled in such a way as to avoid any contamination.

Stability of the working reagent

The **working reagent** is stable for 4 weeks at 2 - 8 °C.

## Sample preparation

- Wine can be analyzed directly.
- Use liquid, clear and nearly neutral samples directly or after dilution into the relevant measuring range (< 300 mg/L).
- 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) |
|---|---|---|
| Reagent 1+2 | 2000 µL | 2000 µL |
| Sample | - | 30 µL |
| Reagent 4/5 (Standard) | 30 µL | - |
| Mix gently each cuvette and incubate for 5 minutes at 37 °C. Then add: | | |
| Reagent 3 (LDH) | 400 µL | 400 µL |
| Incubate 1 minute and read the Abs1 absorbance of the Standard (ST) and Sample (S). Wait another 3 minutes exactly and read the Abs2 absorbance of the Standard (ST) and Sample (S). | | |

**Note:** Because of the precise timing of A1 after 1 min and A2 after another 3 min, it is very challenging to perform this test on a manual photometer. This test kit and application have been validated only on automated analyzers.

## Calculation

Calculate for each 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 $\Delta Abs_{ST}$ 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 $Abs_{CAMP}$ value found on the calibration curve to determine the concentration of the analyzed samples.

Please note: this calculation via a calibration curve is performed automatically when using an automated analyzer.

## Performance data

- No interference is known.
- Linearity of method:
  The test is linear between 80 and 300 mg/L. For concentrations greater than 320 mg/L, it is recommended to dilute the sample 1:2 with distilled water, retest and multiply the result x 2. For concentrations less than 80 mg/L, double the sample volume to bring the concentration to the mentioned range, repeat the determination and divide the result by the concentration factor.
- The sensitivity limit, i.e., the minimum concentration that can be distinguished from zero is 5.5 mg/L.
- A proportional variation of the reaction volumes does not change the result.
- Do not mix reagents from different production lots.
- Support with applications for automatic chemistry analyzers is available upon request.

## References

1. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, W.B. Saunders Co., Philadelphia (2012).
2. Young D.S., Effect of drugs on Clinical Lab. Test, 5th Ed. AACC Press (2000)
3. Berry M.N. et al., Clin. Chem. 35 817 (1989).

## Disclaimer