Colorimetric assay for the determination of Copper in wine
2 x 50 ml (50 assays)

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

Principle

Copper in the sample reacts with DiBr-PAESA [4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylaniline-monosodium salt] under acidic conditions with a reducing agent. The intensity of the coloured complex is proportional to the copper concentration in the sample.

Assay specifications

Wavelength:	580 nm (575 – 600 nm)
Cuvettes:	1.00 cm (glass; plastic)
Temperature:	20 to 37°C
Method:	end point
Reaction:	10 min.
Measurement:	against air or against water
Linearity:	up to 5 mg/L

Reagents

1: Buffer, 2 bottles with approx. 50 ml each (buffer pH 4.9)
NOTE: at <10°C might present a precipitate; always dissolve before use by gently swirling and warming at a temperature > 10°C.

2: Chromogen, 2 bottles with approx. 13 ml (Di-Br-PAESA)

3: Standard, liquid approx. 5 mL (Copper, 5 mg/L).

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

- Wine can be used directly
- Use colorless, clear and neutral liquid samples directly if Copper conc. is between 0.25 – 5 mg/L; otherwise, dilute with water to reduce it in this range
- Strongly coloured samples have to be treated with PVPP (polyvinylpolypyrrolidone e.g. 1 g/100 mL sample)
- For application on biochemistry analysers, it is recommended to add PVP (polyvinylpyrrolidone) at a final concentration of 5 g/l into R1 (1.25 ml of a stock solution 200 g/l in each vial)
- Turbid solutions have to be filtered or centrifuged
- Samples containing carbon dioxide have to be degassed
- Acid samples have to be adjusted by adding KOH or NaOH until approx. pH 5 is reached
- Alkaline samples have to be adjusted by adding HCl until approx. pH 5 is reached

Procedure

Pipette into cuvettes:	Reagent blank (RB)	Standard	Samples
Buffer (reagent 1)	2000 µl	2000 µl	2000 µl
Distilled water	200 µl	-	-
Standard (reagent 3)	-	200 µl	-
Sample	-	-	200 µl
Mix carefully. Read the absorbance A_1 after 5 min. at 20 to 37°C, then add:			
Chromogen reagent (reagent 2)	500 µl	500 µl	500 µl
Mix carefully. Read the absorbance A_2 after 10 min. at 20 to 37°C. The colour is stable 30 min. at room temperature.			

Calculation

$\Delta A = (A_2 - df \times A_1)_{\text{sample or standard}} - (A_2 - df \times A_1)_{RB}$

with df = dilution factor of the optical densities by reagent volumes:
df = (sample volume + R1) / (sample volume + R1 + R2) = 0.815

$$\text{and } C_{sample}\,[mg/L] = \frac{c_{standard}\,[mg/L]}{\Delta A_{standard}} \times \Delta A_{sample}$$

Since the concentration of the standard is fixed at 5 mg/L, this gives the following calculation formula:

$C_{sample}\,[mg/L] = 5 \times (\Delta A_{sample} / \Delta A_{standard})$

Notes

1. For concentrations higher than the limit of linearity, dilute the sample with distilled water in the mentioned ranges; repeat the determination and multiply the result by the dilution factor.
2. Use one way cuvettes or very clean tubes washed with diluted HCl and distilled water.
3. Specificity: this test is specific for Copper, no interferences were detected.

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