Colorimetric method for wines, food and beverages
3 x 70 mL R1 + 1 x 5 mL R2
(105 assays for manual application)

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

Principle

The method is based on the ability of the proteins in the sample to react with the chromogen (pyrogallol): a coloured complex is created, whose colour intensity is proportional to the concentration of proteins present in the sample. Using the standard contained in the kit, a calibration curve is measured and samples are refer to it. The calibration curve must always be repeated at each change of batch, reagent and/or calibrator.

Assay specifications

Wavelength:	600 nm (570 - 620 nm)
Path width:	1 cm
Temperature:	37 °C
Method:	endpoint
Blank:	against Blank Reagent
Linearity:	up to 4000 mg/L

Reagents

#1: R1 - Chromogen (Pyrogallol Red), < 2 µmol/L: 3 bottles approx. 70 mL

#2: R2 - CAL (Protein solution = Concentration value shown on the label, NaN_3 < 0.1%): 1 bottle approx. 5 mL

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 should be supplied on request.

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

Stability of reagents

The reagents are stable until the expiration date indicated on the label, if stored in their intact primary container between +2 °C and +8 °C, not exposed to thermal sources and/or pressure changes, and provided that they have not been contaminated during their use. If the primary container is damaged, dispose of it.

Stability after the first opening

The product is stable up to the expiry date mentioned on the labels after the first open if stored at 2 - 8 °C.

Sample preparation

- Directly use clear, transparent and fairly neutral samples, whose protein concentration is within the measurement range (up to 4000 mg/L, see test performance); otherwise, dilute with distilled water.
- When necessary, use the classic preparation methods for enzymatic/colorimetric tests:
  - Filtration or centrifugation for turbid samples
  - Elimination of carbon dioxide for samples containing carbon dioxide
  - Neutralization at pH 8 for very acidic or alkaline samples

Test procedure

	Reagent Blank (B/R)	Sample (S)	Standard (ST)
Reagent 1	2000 µL	2000 µL	2000 µL
Sample	-	20 µL	-
Standard	-	-	20 µL
Distilled water	20 µL	-	-
Mix gently, incubate for 10 min at 37 °C. Read the absorbance. The final colour is stable for at least 30 minutes.			

Calculation of results

Choice 1: The reagent blank is subtracted from each sample ($Abs_S - Abs_{R/B}$) and standard ($Abs_{ST} - Abs_{R/B}$). This allows to calculate the concentration of the samples according to the following formula:

$$C_{sample}\,[mg/L] = \frac{Abs_S - Abs_{R/B}}{Abs_{ST} - Abs_{R/B}} \times C_{ST}\,[mg/L]$$

Choice 2: Dilute the standard to have several calibration points. Report for each standard point the ΔA_{ST} values against the standard concentration to construct the calibration curve. The calibration curve must always be repeated at each change of batch, reagent and/or calibrator. Report each ΔA_S value found on the calibration curve to determine the concentration of the analyzed samples.

Further calculations

If the sample has been diluted, multiply the result by the dilution factor.

For solid samples with water extraction:

$$\text{Contents}\,[g/100\,g] = \frac{C\,[g/L]}{Weight_{extraction}\,[g/L]} \times 100$$

Performance data

1. There is no known interference.
2. Linearity of the method: the test is linear up to 4000 mg/L. However, for total protein concentrations above 4000 mg/L, it is recommended to dilute the sample with distilled water, retest and multiply the result by the dilution factor.
3. Method Sensibility (LoD): The sensitivity limit, i.e. the minimum concentration that can be distinguished from zero is 11 mg/L.
4. A proportional change in reaction volumes does not change the result.
5. Do not mix Reagents from different production batches together.

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).

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