Colorimetric assay for the determination of wine and must
Test combination for 100 determinations

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

1. Test principle

Under acidic conditions, tartaric acid reacts with a vanadium salt and produces a colored complex (meta-pervanadyl-tartrate). The amount of this chromogen is stoichiometrically related to the amount of tartaric acid present in the sample. It is measured on a spectrophotometer at 520 nm.

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 100 determinations. The number of determinations for automated processing is increased by a multiple; however it depends on the device.

- Reagent 1: 2 x 80 mL with buffer
- Reagent 2: 2 x 25 mL with chromogen
- Decolorant: 1 x 20 mL
- Calibrator: 1 x 5 mL with standard (tartaric acid content: 5 g/L)

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

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 safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- **Note:** The general safety rules for the work in chemical laboratories should be applied. The reagent 1 (containing acetic acid) and the decolorant (containing hypochlorite) should not be mixed together, because they might form chlorine gas (Cl_2). During processing of more than 10 cuvettes, a slight smell of chlorine could appear, so that work should be done under a hood or with sufficient ventilation. After use the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.
- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Wine samples can be used directly.
- Storage of wine samples at 4 °C for a longer time may induce precipitation of tartar, which reduce the measurable amount of tartaric acid in the sample.
- Use colorless and liquid sample solutions (with concentrations between 0.2 - 4 g/L) directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Filter or centrifuge turbid solutions; do not use charcoal to clear red wines.
- Degas samples containing carbonic acid.

4. Assays performance

Wavelength: 520 nm
Light path: 1.00 cm (glass; plastic)
Temperature: 20 - 37 °C (during the measurement)
Method: end point
Reaction time: 5 + 10 min
Measurement: against air (without cuvette) or water
Measuring range: 0.2 - 4 g/L

Pipette into cuvettes	Reagent blank (RB)	Calibrator	Sample / control
Sample (wine, must)	-	-	500 µL
Calibrator	-	100 µL	-
Bi-distilled water	500 µL	400 µL	-
Decolorant	200 µL	200 µL	200 µL
Mix* and incubate for 2 - 3 min. Then addition of:			
Reagent 1 (buffer)	1500 µL	1500 µL	1500 µL
Mix* and incubate for 5 min at 25 - 37 °C. Read the absorbance A_1, then addition of:			
Reagent 2 (chromogen)	250 µL	250 µL	250 µL
Mix and incubate at 25 - 37 °C until the end of the reaction** (10 min). Read the absorbance A_2 (the color is stable during approx. 30 min).			

* The cuvettes must be mixed thoroughly; otherwise, bad recovery and reproducibility results can occur. We recommend using spatulas and mixing one by one. Red wines turn to yellow after mixing with decolorant.

** Air bubbles can appear (Cl_2). In this case, they must be removed with spatulas just before measuring absorbances.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of tartaric acid

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

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume} + H_2O + R1 + \text{decolorant}}{\text{sample volume} + H_2O + R1 + R2 + \text{decoloran}} = 0.898$$

$$C_{sample}\ [g/L] = \frac{C_{calibrator}\ (g/L)}{\Delta A_{calibrator}} \times \Delta A_{sample}$$

Since the concentration of the calibrator is 5 g/L, but the calibrator volume is reduced by 1:5, this gives the following calculation formula:

$$C_{sample}\ [g/L] = \frac{\Delta A_{sample}}{\Delta A_{calibrator}}$$

5.2. Controls

Controls or reference samples should be carried along for quality control during each run.

6. Performance data

6.1. Specificity

The test is specific for D-tartaric acid and L-tartaric acid. Meso-tartaric acid does not react.

6.2. Interferences

Malic acid and lactic acid do not disturb the color reaction up to 5 g/L. If the concentration is higher, recovery might decrease to 80 %.

If the wine is very dark red, the decolorant will not be able to decolorize the sample to yellow; in this case, dilute the wine 1:2 with water and then decolorize again.

6.3. Linearity, measuring range & sensitivity

Check linearity of the spectrophotometer by preparing calibrators with concentrations of 1, 2, 3 and 4 g/L using the calibrator solution delivered with this test kit. Do not calculate sample concentrations outside the linear range.

Sensitivity: in the manual procedure, the lowest detection limit is around 0.1 g/L (ΔA = 0.050).

Note:

Application recommendations for automated analysers are available on request.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Color* Validation report
- Enzytec™ *Color* Excel template for result calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

9. Disclaimer

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