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

For *in vitro* use only
Store between 2 – 8 °C (36 – 46 °F)

1. Test principle

Under acidic conditions, tartaric acid reacts with a vanadium salt (vanadate) and produces a colored complex (metapervanadyl 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 50 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 standard with 5 g/L tartaric acid

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 – 25 °C / 68 – 77 °F) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

- Sample preparation for manual and automated testing is the same.
- Samples solutions should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with distilled water to a concentration within the measuring range (see performance data).
- Wine samples can be used directly.
- Storage of wine samples at 4 °C (39°°F) for a longer time may induce precipitation of tartar, which reduce the measurable amount of tartaric acid in the sample.
- For turbid test samples (e.g., must): Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained. **Do not use charcoal to clear red wines!**
- Degas samples containing carbon dioxide (e.g., beer) by stirring in a beaker, centrifuging, or using a short ultrasonic pulse (10 s).

4. Manual test procedure

Wavelength:	520 nm
Temperature:	23 – 37 °C (73 – 99°°F)
Photometer alignment:	against air (without cuvette)
Measuring range:	0.2 – 5 g/L

	Reagent blank	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 minutes**. Then addition of:			
Reagent 1 (buffer)	1500 µL	1500 µL	1500 µL
Mix* and incubate for **5 minutes** at preferably **25 °C (°77°°F)**. **Read** the absorbance $\mathbf{A_1}$, then addition of:			
Reagent 2 (chromogen)	250 µL	250 µL	250 µL
Mix and incubate preferably at **25 °C (°77°°F)** until the end of the reaction** (**10 minutes**). **Read** absorbance $\mathbf{A_2}$ (the color is stable for approx. 30 minutes).			

4.1 Important notes for assay procedure

- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- 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.
- Specified incubation times were validated at 25 °C (77 °F). The test may generally perform within a range between **23 – 37 °C (73 – 99 °F)**.
- If the cuvettes are not mixed thoroughly before the first incubation*, poor recovery and thus unreliable or non-reproducible results may occur.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance. Red wines turn yellow after mixing with decolorant.
- Air bubbles can appear (Cl_2) during the second incubation**. In this case, they must be removed with spatulas just before measuring absorbances.
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.
- Always wait for the reaction to end or for the absorbance to stabilize (at least during the first test runs or validation). If the absorbance has not stopped after the recommended incubation time, continue measuring at 5-minute intervals, for example, until a constant absorbance value is reached.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of tartaric acid

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

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

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

The following calculation formula is derived from the fact that the concentration of the calibrator is 5 g/L, but the calibrator volume is reduced by a factor of 1:5.

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

5.2. Controls

Control or reference samples should be included in each run for quality control purposes.

As a certified reference material we recommend:

- TraceCERT®43484 Supelco / Tartrate Standard for IC / 1000 mg/L tartrate in water; Sigma # 43484

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

Linearity is given up to 5 g/L tartaric acid (500 µL sample). The recommended measurement range is 0.2 – 5 g/L.

Check linearity of the spectrophotometer by preparing calibrators with concentrations of 1, 2, 3 and 4 g/L (dilute the delivered calibrator solution with water; 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).

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Color* Tartaric acid Validation report
- Enzytec™ *Color* Tartaric acid Excel template for results
- Enzytec™ *Color* Tartaric acid Technical Information
- Enzytec™ *Liquid* Sample preparation guide
- 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. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

Upon request, we offer the following services, among others:

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

10. Disclaimer

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