

Colorimetric assay for wine and must
2 x 80 ml R1 + 1 x 25 ml R2 (100 assays)

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

Principle

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

Assay specifications

Wavelength: 520 nm (505 – 520 nm)
Light path: 1.00 cm (glass; plastic)
Temperature: 20 to 37°C
Method: end point
Reaction: 10 minutes
Measurement: against air or against water
Linearity: 0.2 – 4 g/L (see also note 1)

Reagents

- # 1: Reagent 1 (buffer), 2 bottles with approx. 80 ml
- # 2: Reagent 2 (Chromogen), 1 bottle with approx. 25 ml.
- # 3: Decolorant, 1 bottle with approx. 20 ml
- # 4: Calibrator, 1 bottle with approx. 5 ml (Tartaric acid 5 g/l)

All reagents are ready for use. They are stable at 2-8 °C up to the expiry date shown on the package if not contaminated during handling.

Let the reagents reach the laboratory temperature (20 - 25°C) before use. Mix kindly before pipeting. Close immediately after handling. The reagents have to be used properly, to avoid contamination.

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 (Cl₂). During processing of more than 10 cuvettes, a slight smell of chlorine could appear, so susceptible persons should work 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

- Wine can be used directly.
- Use clear and liquid samples directly if Tartaric acid concentration is between 0.2 – 4 g/l; otherwise, dilute with water (in the cuvette) to reduce it in this range.
- Turbid solutions have to be filtered or centrifuged; do not use charcoal to clear red wines
- Samples containing carbon dioxide have to be degassed
- Storage of wines at 4°C for a longer time may induce precipitation of tartar which reduce the measurable amount of tartrate in the sample

Procedure for wines

Pipette into cuvettes:	Reagent Blank (RB)	Calibrator	Samples
Sample (wine)	-	-	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 add:			
Reagent 1 (buffer)	1500 µl	1500 µl	1500 µl
Mix* and incubate for approx. 5 min. at 25 – 37°C. Read the absorbance A ₁ , then add:			
Reagent 2 (chromogen)	250 µl	250 µl	250 µl
Mix and incubate at 25-37°C until the end of the reaction** (approx. 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 (Cl₂). In this case they must be removed with spatulas just before measuring absorbances

Calculation of results

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

with df = dilution factor of the optical densities by reagent volumes:
df = (sample + H₂O + R1 + decolorant) / (sample + H₂O + R1 + decolorant + R2) = 0.898

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

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:

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

Notes

1. 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.
2. The test is specific for D-tartaric and L-tartaric acid. Meso-tartaric acid does not react.
3. 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%.
4. Sensitivity: in the manual procedure, the lowest detection limit is around 0.1 g/l (ΔA = approx. 0.050)
5. Examples of applications for automated analysers are available on request.
6. 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