Colorimetric assay for wine
2 x 100 ml R1 + 1 x 10 mL R2 (100 assays)

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

## Principle

The total sulphite content in wine is measured at a pH value where all sulphite is liberated from its binding partners (e.g. acetaldehyde) and reacts with a specific colour reagent. The amount of this chromogen is stochiometrically related to the amount of sulphite present in the sample. It is measured on a spectrophotometer at 340 nm.

## Assay specifications

| | |
|---|---|
| Wavelength: | 340 nm (± 5 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: | 10 – 300 mg/L (total sulphite) |

## Reagents

# 1: Reagent 1 (buffer): 2 bottles with approx. 100 mL each

# 2: Reagent 2 (chromogen): 1 bottle with approx. 10 mL

# 3: Calibrator: 1 bottle with approx. 5 ml ($SO_2$ = 300 mg/l)

All reagents are ready for use. These reagents 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 working temperature (+20 to +37°C) before use.**

The general safety rules for working in chemical laboratories should be applied. After use the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

## Sample preparation

- Wine can be used directly.
- **Be aware that sulphur dioxide is volatile and losses can occur.**
- Use clear liquid samples directly if sulphite concentrations are within the linear range of the assay; otherwise, dilute with the buffer (reagent 1).
- Turbid solutions have to be filtered or centrifuged.
- Storage of opened wines is not possible; always close the bottle or container after pipetting a wine sample

## Procedure for total sulphite in wines

| Pipette into cuvettes: | Reagent Blank (RB) | Calibrator | Samples |
|---|---|---|---|
| Reagent 1 (buffer) | 1900 µL | 1900 µL | 1900 µL |
| Calibrators (300 mg/L) | - | 100 µL | - |
| Sample | - | - | 100 µL |
| Dist. Water | 100 µL | - | - |
| Mix cuvettes*, read absorbance $A_1$ at 340 nm after 2 – 3 min | | | |
| Reagent 2 (chromogen) | 100 µL | 100 µL | 100 µL |
| Mix cuvettes*. Read the absorbance $A_2$ at 340 nm after 10 to 15 minutes | | | |

* Use spatulas for **intensive mixing**, otherwise low precision occurs (keep same spatula for each cuvette until the end)

## Calculation of results

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

with df = dilution factor of the optical densities by reagent volumes:
df = (sample + R1) / (sample + R1 + R2) = 0.952

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

Since the concentration of the calibrator is 300 mg/l, this gives the following calculation formula:
$C_{sample}\,[mg/L] = 300 \times (\Delta A_{sample} / \Delta A_{calibrator})$

Quantitation below 10 mg/l is not recommended, report the results as "< 10 mg/l".
In case of red wines, **add 20 mg/L** to results above 10 mg/l. Results below 10 mg/l must be reported as being below 30 mg/l.

## Notes

1. Interferences: compounds containing free thiols or thiol reactive compounds
2. Sensitivity: in the manual procedure, the detection limit is around 5 mg/L
3. Examples of applications for automated analysers are available on request.
4. Use only fresh bi-distilled water to dilute the calibrators. Otherwise losses will occur and samples are overestimated.

## Example of results

The linearity of the photometer should be checked from time to time by dilution series of the calibrator. It is also possible to buy Sodium metabisulfite ($Na_2S_2O_5$) as additional calibrator or as quality control. But it is not stabilized as the kit calibrator is, so it should be prepared freshly each day.