

Colorimetric assay for wine, must and other food samples
2 x 100 mL R1 / 2 x 25 mL R2 / 3.5 mL calibrator (100 assays)

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

Principle

The free sulphite is determined with a specific colour reagent at an acidic pH value. The amount of chromogen is stoichiometrically related to the amount of sulphite present in the sample, and is measured on a photometer at 340 nm.

Assay specifications

Wavelength: 340 nm (± 5 nm)
Light path: 1.00 cm (glass or plastic)
Temperature: 20 to 37°C
Method: end point
Reaction: 10 min (20 - 25°C) or 5 min (37°C)
Measurement: against air or against water
Linearity: 10 – 300 mg/L (free sulfite)

Reagents

The reagents are ready-to-use.

- # Reagent 1 (buffer): two vials ≥ 100 mL
- # Reagent 2 (chromogen): two vials ≥ 25 mL
- # Calibrator (SO₂ equivalent = 50 mg/l): one vial ≥ 3.5 ml

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C. Do not freeze the reagents. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

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 further hazardous substances. For hazard notes on the contained substances, please refer to the appropriate material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

- Sulphur dioxide is volatile and sensitive to oxidation, so losses can occur
- The samples must be stored in a closed container, warmed up to room temperature and opened shortly before testing
- Use clear and liquid samples directly, turbid solutions have to be centrifuged (filtering would cause losses of SO₂)
- Wine can be used directly

Assay procedure

Pipette into cuvettes:	Reagent Blank (RB)	Calibrator	Samples
Reagent 1 (buffer)	2000 µL	2000 µL	2000 µL
Calibrator (50 mg/L)	-	100 µL	-
Sample	-	-	100 µL
Dist. Water	100 µL	-	-
Mix*, incubate 3 min, read absorbance A ₁ , then add:			
Reagent 2 (chromogen)	500 µL	500 µL	500 µL
Mix* and incubate 10 min (20 – 25 °C) or 5 min (37°C) . Read absorbance A ₂ .			

* Use spatulas for mixing

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 + R1) / (sample + R1 + R2) = 0.808

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

Since the concentration of the calibrator is 50 mg/l, this gives the following calculation formula:

$$C_{\text{sample}} [\text{mg/L}] = 50 \times (\Delta A_{\text{sample}} / \Delta A_{\text{calibrator}})$$

Notes

1. The calibrator has been specifically designed for use with this colorimetric assay, and not for other reagents.
2. It is necessary to control each run with a quality control. For this purpose, it is recommended to use sodium metabisulphite (Na₂S₂O₅), which seems to be more stable than sodium sulphite (Na₂SO₃). But it is not stabilized as the kit calibrator, so it should be prepared **freshly each day**. Do not use glass but plastic vials like Eppendorf cups.
3. Use only fresh bi-distilled water to prepare calibrators and controls, otherwise oxidation of SO₂ could occur
4. Examples of applications for automated analysers are available on request.

Test performance

Specificity

The test is specific for SO₂ / SO₃. Interferences were observed with compounds containing free thiols, thiol-reactive compounds and sodium nitrite.

Linearity and measuring range

example of results

SO ₂ (mg/L)	A1	A1*df	A2	Δ A	minus Blank
0	0.050	0.040	0.196	0.156	0.000
Calibrator	0.047	0.038	0.413	0.375	0.219
150	0.050	0.040	0.885	0.845	0.689
300	0.050	0.040	1.558	1.518	1.362

Even if the calibrator is limited at 50 mg/l, the test is linear up to 300 mg/l and results can be extrapolated up to that concentration.

Sensitivity

The Limit of Detection (LoD) and Limit of Quantification (LoQ) where determined according to the method DIN 32645:2008-11:

$$\text{LoD} = 4.0 \text{ mg/l} \qquad \text{LoQ} = 7.0 \text{ mg/l}$$

Quantitation below 10 mg/l is not recommended, report the results as "< 10 mg/l".

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