Colorimetric assay for the determination of total sulfite in foodstuffs and other sample materials
Test combination for 100 determinations

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

1. Test principle

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

The reaction can be represented by the following chemical reaction:

SO_3^{2-} + chromogen (no absorption at 340 nm) $\longrightarrow$

S^--chromogen$_{modified}$ (absorption at 340 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 100 mL with buffer
- Reagent 2: 2 x 25 mL with chromogen
- Calibrator: 1 x 3.5 mL (SO_2 = 150 mg/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

- Samples must be stored in a closed container and opened shortly before testing.
- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly to a concentration within the measuring range (see performance data).
- Centrifuge turbid solutions.
- Degas samples containing carbonic acid by a short ultrasonic shock at 0 °C (ultrasonic device filled with ice cubes and distilled water).
- Wine can be used directly.

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 3 - 300 mg/L

	Reagent blank	Calibrator	Sample / control
Reagent 1	2000 µL	2000 µL	2000 µL
Calibrator	-	100 µL	
Sample / control	-	-	100 µL
Dist. water	100 µL	-	-
Mix, incubate for 3 min at 20 - 37 °C. Read absorbance A_1, then addition of:			
Reagent 2	500 µL	500 µL	500 µL
Mix, incubate for 5 min at 20 - 37 °C and read absorbance A_2.			

The reagent blank value must be determined once for each run and subtracted from each sample result.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of SO_2-total

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

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume + R1}}{\text{test volume}} = 0.808$$

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

Since the concentration of the calibrator is 150 mg/L, this gives the following calculation formula:

$$C_{sample}\ [mg/L] = 150 \times (\Delta A_{sample} / \Delta A_{calibrator})$$

Notes:

1. Sulfur dioxide (SO_2) is volatile and sensitive to oxidation, so losses can occur.
2. When iodometric titration is performed via a simple alkaline treatment (without distillation), the method will measure all reducing substances in addition to the SO_2. The colorimetric method measures only SO_2, so it is normal to obtain lower results.
3. Use only fresh bi-distilled water to dilute calibrators and controls, to avoid SO_2 oxidation.
4. Examples of applications for automated analysers are available on request.

5.2. Controls & acceptance criteria

Control or reference samples should be carried along for quality control during each run. For this purpose, we recommend a daily-prepared solution of sodium disulfite ($Na_2S_2O_5$) in water and 1 g/L citric acid (for stabilization).

Furthermore, thiosulfate (or one of its salts) can also be used. This has the advantage that it is not oxidized by oxygen after opening. Sodium thiosulfate, for example, is commercially available as a ready-to-use solution.

The recovery of aqueous sulfite control solutions should be 100 ± 5 %.

6. Performance data

6.1. Specificity

0The test is specific for SO_2 / the sulfite anion SO_3^{2-}.

6.2. Interferences

Interferences were observed with compounds containing free thiols and thiol-reactive compounds.

6.3. Linearity, measuring range & sensitivity

Even if the calibrator is limited at 150 mg/L, the test is linear up to 300 mg/L and results can be extrapolated up to that concentration.

The recommended measuring range is between 3 and 300 mg/L (sample volume of 100 µL).

Example of results

SO_2 (mg/L)	A_1	A_1 * df	A_2	ΔA	minus blank
0	0.050	0.040	0.108	0.067	0.000
50	0.048	0.039	0.325	0.286	0.219
Calibrator	0.049	0.040	0.777	0.737	0.670
300	0.050	0.040	1.408	1.368	1.301

The limit of detection (LoD) was determined for a sample volume of v = 100 µL according to method DIN 32645:2008-11. This results in an LoD of 1.5 mg/L. The limit of quantification (LoQ) is 3 mg/L.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results 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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