

Colorimetric assay for the determination of SO₂ 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 free sulfite is determined with a specific colour reagent at an acidic pH value. The amount of chromogen is stoichiometrically related to the amount of sulfite present in the sample, and is measured on a photometer at 340 nm.

The reaction can be represented by the following chemical reaction:
SO₃²⁻ + chromogen (no absorption at 340 nm) →
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 (50 mg/L SO₂-equivalent)

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

- 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.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- The samples must be stored in a closed container, warmed up to room temperature and opened shortly before testing.

4. Assays performance

Wavelength: 340 nm
Temperature: 25 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 7 - 300 mg/L (free SO₂)

	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 25 - 37 °C. Read absorbance A ₁ , then addition of:			
Reagent 2	500 µL	500 µL	500 µL
Mix, incubate for 10 min at 25 °C or 5 min at 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 free SO₂

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

df: Dilution factor
RB: Reagent blank

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

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume is increased, the test system may be affected. In general, this must be checked depending on the matrix.

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

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. Sulfur dioxide is volatile and sensitive to oxidation, so losses can occur.
2. The calibrator has been specifically designed for use with this colorimetric assay, and not for other reagents.
3. It is necessary to control each run with a quality control. For this purpose, it is recommended to use sodium metabisulfite (Na₂S₂O₅), which seems to be more stable than sodium sulfite (Na₂SO₃). But it is not stabilized as the kit calibrator, so it should be **prepared freshly each day**. Do not use glass vials but plastic vials like Eppendorf cups.
4. Use only fresh bi-distilled water to prepare calibrators and controls, otherwise an oxidation of SO₂ could occur.
5. 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 a sulfite salt (e.g. sodium metabisulfite Na₂S₂O₅) in water and 1 g/L citric acid (for stabilization). The appropriate sulfite concentration must be calculated according to the molecular weight of the salt used.

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 & side activities

The test is specific for SO₂ / the sulfite anion SO₃²⁻. Side activities could not be determined.

6.2. Interferences

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

6.3. Linearity, measuring range & sensitivity

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. The recommended measuring range is between 7 and 300 mg/L (sample volume of 100 µL).

Example of results

SO ₂ (mg/L)	A1	A1 * df	A2	ΔA	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

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 4.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile and is 7.0 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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