Colorimetric assay for the determination of free $SO_2$ in foodstuffs and other sample materials
For *in vitro* use only
Test combination for 100 determinations

Store between 2 – 8 °C (36 – 46 °F)

This test was validated using selected samples of the following matrices: wine.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user.

## 1. Test principle

Free $SO_2$ is measured using a specific dye at an acidic pH value. At this pH, the chromogen reacts with the sulfite anion $SO_3^{2-}$. The amount of chromogen that has reacted is proportional to the concentration of sulfite in the sample and is measured based on its absorption at 340 nm.

The reaction can be represented by the following chemical equation:

$SO_3^{2-}$ + chromogen $\longrightarrow$ $S^-$-chromogen $_{modified}$

## 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 12.5 mL with chromogen
- Calibrator: 1 x 3.5 mL with 50 mg/L $SO_2$-equivalent

### *2.2. Reagent preparation*

The reagents are ready-to-use and be allowed to reach room temperature (20 – 25 °C / 68 – 77 °F) before use. Do not interchange components between kits of different batches.

### *2.3. Storage & stability*

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

### *2.4. Safety & disposal*

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

## 3. Sample preparation

- Sample preparation for manual and automated testing is the same.
- Samples solutions should be brought to room temperature before measurement.
- Use liquid, clear sample solutions directly or after dilution with distilled water to a concentration within the measuring range (see performance data).
- Use only fresh distilled water to dilute samples, calibrators, or controls to prevent $SO_2$ oxidation.
- For turbid test samples (e.g., sauerkraut juice, pineapple juice): Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained. Use 100 µL (or more if necessary; fruit juices undiluted, fermented vegetable juice should be diluted before measurement).
- If necessary, decolorize strongly colored samples (such as wine and juices) with polyvinylpolypyrrolidone (PVPP, e.g., 1 g per 100 mL of sample). Stir or shake the sample for 1 minute. Then filter or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- Degas samples containing carbon dioxide (e.g., beer) using a short ultrasonic pulse (10 s); filter if necessary if the extract is not clear, and use 100 µL in the test.
- **Important:** Carrez clarification cannot be used during sample preparation for sulfite determination.

## 4. Manual test procedure

| | |
|---|---|
| Wavelength: | 340 nm |
| Temperature (measurement): | 25 – 37 °C (77 – 99 °F) |
| Photometer alignment: | against air (without cuvette) |
| Measuring range: | 7 – 300 mg/L (free $SO_2$) |

| | **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 Minutes at 25 – 37 °C (77 – 99 °F)**. **Read absorbance $A_1$**, then addition of: | | | |
| **Reagent 2** | 500 µL | 500 µL | 500 µL |
| Mix, incubate for **10 Minutes at 25 °C (°77 °F)** or **5 minutes at 37 °C* (99 °F)** and **read absorbance $A_2$**. | | | |

***Important notes for assay procedure***

- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- Specified incubation times were validated at 25 °C (99 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- * Please refer to Chapter 5.3
- $SO_2$ is volatile, reactive, and easily oxidized by atmospheric oxygen, which can result in losses. These properties of the analyte must be taken into account during sample preparation and test performance, or when comparing data from different methods.
- The calibrator was developed specifically for this test and must not be used in other methods.
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary.

## 5. Calculation of results

### 5.1. *Concentration of free $SO_2$ in sample solutions*

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume [mL]} + \text{volume R1 [mL]}}{\text{reaction volume [mL]}} = 0.808$$

The specified df value of **0.808 applies to a base application of 100 µL**. An increase in sample volume is possible (max. 1000 µL). **While keeping reagent volumes unchanged**, this requires **conversion of the reagent dilution factor (df)** accordingly.

Increasing the sample volume may influence test performance. This must generally be checked depending on the matrix. **The reagent blank value must be adjusted to the changed sample volume.**

The test is calibrated using a standard. The optical density measured for a sample is compared to this standard, allowing the concentration to be calculated:

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

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

$$C_{\text{sample}}\,[mg/L] = 50\ mg/L \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{calibrator}}} \times F$$

### 5.2. *Calculation of solid samples*

When analyzing solid and semi-solid samples that have to be weighed in for the extraction of the sample, the content is related to the sample weight:

$$\text{Content}_{\text{sulfite}}\,[g/100\ g] = \frac{C_{\text{sulfite}}\,[g/L\ \text{sample sol.}]}{\text{weight}_{\text{sample}}\ \text{in g/L sample sol.}} \times 100$$

### 5.3. *Controls & acceptance criteria*

Control or reference samples should be carried along for quality control during each run. For this purpose, we recommend a solution prepared from a sulfite salt. Since this solution is not stabilized, unlike the supplied calibrator, it must be **freshly prepared each day** (use plastic tubes, not glass tubes). The appropriate sulfite concentration must be calculated based on the molecular weight of the salt used.

Sodium thiosulfate is commercially available as a ready-to-use solution.

The recovery of *aqueous* sulfite control solutions should be 100 ± 5%. If required, the A2 incubation time may need to be adapted to the specific laboratory conditions.

## 6. Performance data

### 6.1. *Specificity & side activities*

The test is specific for $SO_2$ and the sulfite anion $SO_3^{2-}$. No side activities were detected.

### 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 7 – 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 | 0.196 | 0.156 | 0.000 |
| Kalibrator | 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 according to the DIN 32645:2008-11 method in a buffered aqueous solution. For a sample volume of 100 µL, the calculated LoD is 4.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile. For a sample volume of 100 µL, the calculated LoQ is 7.0 mg/L.

## 7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* $SO_2$-Free Validation Report
- Enzytec™ *Liquid* $SO_2$-Free Excel template for results
- Enzytec™ *Liquid* $SO_2$-Free Technical Information
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:

https://eifu.r-biopharm.com/

## 8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

## 9. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Workflow analysis
- Data & results analysis
- Customer workshops & webinars
- Automation: application support & technical service

## 10. Disclaimer

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