Enzymatic assay for the determination of sulfite (as total SO_2) in foodstuff and other sample materials
30 mL buffer / 0.4 mL NADH-POD / 1.6 mL SUOX (30 assays)

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

Principle

Enzymatic assay using NADH-peroxidase (NADH-POD) and sulfite oxidase (SUOX). The consumption of NADH is measured at 340 nm:

$SO_3^{--} + O_2 + H_2O \xrightarrow{SUOX} SO_4^{--} + H_2O_2$

$H_2O_2 + NADH + H^+ \xrightarrow{NADH\text{-}POD} 2\ H_2O + NAD^+$

Reagent preparation

Four reagents are present:
Vial 1: Buffer (30 mL, TEA 0.8 M, NaN_3 0.02 %)
Vial 2: NADH tablets (0.4 mg NADH each)
Vial 3: Suspension with NADH-POD (0.4 mL, 14.5 U/mL)
Vial 4: Suspension with SUOX (1.6 mL, 2.5 U/mL)

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C. For long-term stability store vial 2 desiccated at -30 to -15 °C. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

Two reagents must be prepared as following:

Working solution 1 + 2: Dissolve 1 tablet (vial 2) per 1 mL of buffer (vial 1), depending on the number of samples tested. This working solution is stable for 1 week if stored at 2 - 8 °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 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

- Use liquid and clear samples directly, or after dilution into the relevant measuring range (see test performance).
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Carrez clarification is not allowed for sulfite testing!
- Crush and homogenize solid or semi-solid samples and extract with water; filtrate or centrifuge.
- Since sulfite is volatile, reactive and easily oxidized, please take special care when preparing the samples and performing the analysis. Due to instability of sulfite solutions, samples should be analysed as soon as possible after preparation.

Assay procedure

Wavelength:	340 nm
Optical path:	1 cm
Total volume:	3.060 mL
Temperature:	~ 25 °C
Blank:	against air or against water
Sample solution:	1 - 30 µg/assay

	Reagent blank	Samples
Solution 1+2	1000 µL	1000 µL
Sample	-	100 µL
Dist. water	2000 µL	1900 µL
NADH-POD (vial 3)	10 µL	10 µL
Mix, incubate for 5 min at 20 - 25 °C. Read absorbance A_1 in time, then start the reaction by addition off:		
SUOX (vial 4)	50 µL	50 µL
Mix, incubate at 20 - 25 °C until the end of the reaction (approx. 30 min)*. Read absorbance A_2.		

**If necessary, continue to measure the absorbance at 5 min intervals until the reaction ends, or measure the creep reaction and subtract it.*

Calculation of results

The results are calculated with the Lambert-Beer law.

$\Delta A = (A_1 - A_2)_{sample\ resp.\ standard} - (A_1 - A_2)_{blank}$

$c = (V \times MW \times \Delta A) / (\varepsilon \times d \times v \times 1000)$ [g/L]

$c = (3.060 \times 64.06 \times \Delta A) / (6.3 \times 1.00 \times 0.100 \times 1000)$

$c = 0.311 \times \Delta A$ [g/L]

If the sample has been diluted during preparation, multiply the result with dilution factor.
When analysing samples which are weighed out for the sample preparation, calculate the content from the amount weighted:

$$\text{Content [g/100 g]} = \frac{C_{test}\ [g/L]}{weight_{sample}\ [g/L]} \times 100$$

Test performance

Specificity

Sulfite oxidase reacts with sulfites, isothiocyanates and their glycosides. Organic sulfonic acid compounds can give rise to a degree of creep reaction. Sulfides, thiosulfates, sulfate and organic sulfinic acid compounds do not react under the assay conditions. Purified reagents, such as sodium sulfite, sodium disulfite and potassium disulfite absorb moisture and are easily oxidized. In addition, aqueous solutions are instable. Thus, under these conditions, values below 100 % should be expected.

Interference

L-Ascorbic acid inhibits sulfite oxidase. High concentrations of L-ascorbic acid in the assay react with hydrogen peroxide, which is formed as an intermediary product, and thus produce results that are too low. L-ascorbate must be removed before the determination of sulfite, e.g. by means of ascorbate oxidase.

Measuring range

The recommended measuring range is 1 µg - 30 µg per assay (cuvette). For a volume of 100 µL sample, this means 10 - 300 mg/L. If this range is exceeded, the samples should be diluted with distilled water to a concentration within the measuring range. The dilution factor must be included in the calculation.

Sensitivity

The sensitivity is calculated with the Lambert-Beer law above and thus varies depending on v and ΔA chosen. The minimum ΔA that can be measured in a reproducible way is ΔA = 0.020 (A). The sample volume (v) can be increased up to 2 mL (reduce the water accordingly). The calculation gives following results as example:
- with v = 0.100 mL and ΔA = 0.050, limit = 15 mg/L (routine vol.)
- with v = 0.500 mL and ΔA = 0.050, limit = 3 mg/L (intermediate vol.)
- with v = 2.000 mL and ΔA = 0.020, limit = 0.3 mg/L (maximum vol.)

Quality control

Prepare a quality control freshly each day in 100 mL of citric acid solution (1 g/L) for obtaining 300 mg/L equivalent SO_2:
- for sodium sulfite (Na_2SO_3, 50.8 % SO_2) dissolve 59 mg
- for sodium disulfite ($Na_2S_2O_5$, 67.4 % SO_2) dissolve 44.5 mg
- for potassium disulfite ($K_2S_2O_5$, 57.6 % SO_2) dissolve 52 mg

Supplementary technical notes

In case of difficulties that may arise, the implementation of these recommendations may contribute to an improved quality of results:

1. Working solution 1+2: Dissolve 1 tablet (bottle 2) in 1 mL buffer (bottle 1), depending on the number of samples examined. Recommendation: Allow the working solution to settle for at least 30 minutes before use in the test. This will ensure that the NADH tablets are completely dissolved and you obtain an even more stable working solution. This can have a positive effect on the A_1 measurement.

2. Since sulfite is volatile, reactive and easily oxidized, special care should be taken when preparing samples and performing the analysis.
   Recommendation: Measure the blank in duplicate and subtract the average of these two blanks from your samples. This will give you an even more accurate blank value, which after subtraction can have a positive effect on the quality of the results of your samples used.

3. Experience shows that the water quality can have an effect on the result quality of enzymatic tests.
   Recommendation: Use only water of the highest quality when running the kit E6275 to ensure the best results. In case of doubt, treat water with activated carbon (stir in 1 g / 100 mL and filter after approx. 3 min).

4. Creeping reactions have an influence on the result quality, especially for samples in the low concentration range. Recommendation: Mix after R2 addition and measure the absorbance values of the solutions (A_2) exactly 30 min after A_1 measurement (as described in the instructions for use). After exactly another 15 min, measure the absorbances of the solutions (A_3) again.

   Since the creep reaction was now measured only during 15 min (between A_2 and A_3), but must be extrapolated to the 30 min incubation time after which the measurement was performed (between A_1 and A_2), subtract the creep reaction by a factor of 2 according to the following calculation:
   $\Delta A = (A_1 - A_2) - 2 \times (A_2 - A_3)$.

   This system allows a fast and systematic measurement of the creeping reaction over A_3 and its automatic subtraction via the excel template at your disposal.

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