Enzytec™ Sulfite (SO_2-total)

Ref. No. E6275

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:

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

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

Reagents 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 NADH-POD (0.4 ml, 14.5 U/ml)
Vial 4: Suspension 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. Do not freeze the reagents. 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.

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.

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

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

c = (3.060 x 64.06 x ΔA) / (6.3 x 1.00 x 0.100 x 1000)

c = 0.311 x ΔA [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:

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

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 (1g/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

Disclaimer

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