CREATIVE BIOMART®
Assay Kit

Sulfite Assay Kit

Product Information

Common Name

Sulfite

Cat.No. Kit-1677

Product Overview

Sulfur dioxide, sulfurous acid and its salts (sulfites) occur in very low concentrations in nature. However, they have been used for a very long time in the industrial production of foodstuffs ("sulfurating"). Sulfur dioxide is used as a preservative in food industry in order to prevent microbial spoilage. The usage of sulfuric acid in the production in wine belongs to the most important techniques in order to improve stability and taste of wine. Sulfite is regarded as being poisonous for cells; in metabolism, it is rapidly oxidized to sulfate and excreted. The sulfite content in foodstuffs is legally prescribed in a number of countries and the content has often to be declared on the label.

Description

Enzymatic method for the determination of sulfite. Based on the spectrophotometric measurement of NADH formed through the combined action of sulfite oxidase (SOD) and NADH-peroxidase (NADH-POD).

Characteristic

Quantity: 30 tests
Reaction volume: 3.06 mL
Range: 0.25-300 mg/L
Detection limit: 0.25 mg/L

Applications

This rapid and simple specific enzymatic method is used for the determination of sulfite (“total SO2”) in foodstuffs such as wine, beer, fruit and vegetables, as well as in pharmaceuticals and water.

Synonyms

Sulfite; UV method; SO2; foodstuffs; NADH; Sulfite oxidase; Sulfite Assay Kit; Sulfite test

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Size

30 tests

Kit Components

Solution 1. Triethanolamine buffer (30 mL, 0.8 M, pH 8.0) and sodium azide (0.02% w/v) as a preservative. Stable for 2 years at 4 °C.
Solution 2. NADH tablets (0.4 mg each). Stable for 5 years at -20 °C. Dissolve 3 tablets per 2 mL of solution 1; use forceps for taking the tablets out.
Suspension 3. NADH-peroxidase (NADH-POD, EC 1.11.1.1; 500 U/mL) (0.4 mL). Stable for 2 years at 4 °C. Swirl bottle before use.
Suspension 4. Sulfite oxidase (SOD, EC 1.8.3.1; 1.0 U/mL) in 3.2 M ammonium sulphate (1.6 mL). Stable for 2 years at 4 °C. Swirl bottle before use.
Solution 5. Sulfite control (2 g sodium sulfite). Stable for 2 years at 4 °C. Dissolve approx. 60 mg of sodium sulfite (30 mg sulfite) in 100 mL of distilled water; store in a well-sealed bottle and use on the day of preparation. This control solution can be used when there is some doubt about the method accuracy.

Features & Benefits

Stable enzyme suspensions
Simple format
Suitable for manual and micro volume formats

Preparation

The amount of sulfite present in the cuvette should range between 0.6 and 30 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield a sulfite concentration between 6 and 300 mg/L. However, the sample volume can vary from 0.10 to 2.00 mL, by replacing water (analyte range from 0.30 to 300 mg/L). To implement this assay use clear, colourless and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (e.g. by filtration); adjust acid samples, which are used undiluted for the assay, to pH 8 by adding sodium or potassium hydroxide solution; adjust acid and weakly coloured samples to pH 8 and incubate for approx. 15 min; measure "coloured" samples (if necessary

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adjusted to pH 8) against a sample blank; treat "strongly coloured" samples that are used undiluted or with a higher sample volume with PVPP; crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water. Since sulfurous acid is volatile, reactive and easily oxidized, please take special care when preparing the samples and performing the analysis.

Examples of sample preparation:

Determination of sulfite in white wine: Use white wine directly for the assay with a sample volume of v = 0.10 mL.

Determination of sulfite in red wine: Adjust 25 mL of red wine to pH 7.5-8.0 with sodium hydroxide (2 M) and dilute to 50 mL with distilled water in a volumetric flask (dilution factor of 2). Incubate for approx. 10 min at 25 °C. Use 0.10 mL for the assay. Determination of sulfite in beer: Open bottle and filter beer sample immediately at 25 °C. Add 0.7 g bentonite to 10 mL beer in a 50 mL beaker, stir for 1 min and filter. Use 1.00 mL of the filtrate for assaying.

Determination of sulfite in jam: Homogenize approx. 100 g jam in a homogenizer for 30 s. Weigh precisely approx. 5 g of the homogenous sample into a 50 mL volumetric flask and add 40 mL double destilled water. Close the volumetric flask and incubate at 60 °C for 5 min. Cool to 25 °C, fill up to the mark with distilled water, mix and filter. Use the clear solution diluted, if necessary, for the assay.

Determination of sulfite in potato products: Mince and homogenize dried potato products using a mortar or mixer. Weigh precisely approx. 5 g of minced and ground potato chips (or approx. 2 g ground dried potato material intended for production of potato dumplings) into a 100 mL volumetric flask and add 80 mL hot (65 °C) distilled water. Close the volumetric flask and shake rigorously for 5 min or stir with magnetic stirrer. Let stand for 15 min. After cooling to 25 °C, fill up to the mark with distilled water, mix and centrifuge (10 min at 4000 rpm). Use the clear solution with a sample volume of up to 0.50 mL or diluted, if necessary, for the assay.

Determination of sulfite in spices and coffee products: Mince and homogenize the spice sample using a mortar or mixer. Weigh precisely approx. 100 mg sample into a 50 mL volumetric flask and add 30 mL distilled water. Close the volumetric flask and incubate at approx. 60 °C for 5 min. Cool to 25 °C, fill up to the mark with distilled water, mix and filter. Use the clear solution diluted, if necessary, for the assay.

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Assay Protocol

Procedure (endpoint analysis)
Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 25 °C
Final volume: 3.060 mL
Sample solution: 0.6-30 µg sulfite/assay (as SO2; in 0.10 - 2.00 mL sample volume)
Read against air (without a cuvette in the light path) or against water
Pipette into cuvettes (mL) Blank Sample
Solution 1+2 1.00 1.00
Sample - 1.00
Distilled water 2.00 1.90
Suspension 3 (NADH-POD) 0.010 0.010
Mix, measure the absorbance of the solutions (A1) after 5 min and start the reaction by addition of
Suspension 4 (SOD) 0.050 0.050
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 20 min)*
Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm.
* if necessary, continue to read the absorbances at 5 min intervals until the reaction ends.
Alternative procedures (micro-volumes)
Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the standard cuvettes pathlength.

Analysis

Determine the absorbance difference for both blank and sample (A1-A2). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining ΔASulfite. The concentration of sulfite (as SO2), based on the ε of NADH at 340 nm (6300 L×mol-1×cm-1), is

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calculated as follows:
C (sulfite) = 0.3111 x ΔASulfite [g/L]
If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor

Sensitivity

The sensitivity of the assay is based on 0.005 AU and a sample volume of 2.00 mL. This corresponds to a sulfite concentration of 0.1 mg/L sample solution when measured at 340 nm. The detection limit of 0.30 mg/L is derived from the absorbance difference of 0.010 (340 nm) and a maximum sample volume of 2.00 mL.

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