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Product Information

Total Sulfite Assay Kit

Catalog Number **MAK366**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Sulfites/sulphites are substances that occur naturally in the human body due to the metabolism of amino acids containing sulfur in their side chains. They are readily oxidized to sulfates via enzymatic reactions and excreted in urine at the rate of 1,000 mg/day. Sulfites are often considered as allergens due to adverse symptoms observed in asthmatic patients. Exogenous sources of sulfites include polluted air, food, and beverages containing sulfur dioxide (SO_2). SO_2 is a molecule that easily reacts with several small compounds including aldehydes, ketones, anthocyanins, cobalamine, thiamine, NAD, flavins, among others. SO_2 also interacts with cysteine residues in proteins and promotes crosslinking. Sulfites are also used as regulated food preservatives/additives/ enhancers in dried fruits, wine, beer, etc. They are considered as GRAS (Generally Recognized as Safe) in food and beverages. In wine, SO_2 reacts with sugars, aldehydes, and anthocyanins. The term sulfite is used for all sulfite-derived molecules: bisulfites, metasulfites, and SO_2. Total sulfite is defined as the sum of bound and free sulfite.

The Total Sulfite Assay Kit is a simple and sensitive assay to detect small concentrations of sulfites in a variety of food and beverage samples. This assay is based on the oxidation of sulfite to sulfate producing a stable signal at 570 nm (A_{570}), which is directly proportional to the amount of sulfite in the sample. This assay is very sensitive and can detect as low as 20 μM of sulfite in a variety of samples.

The kit is suitable for the measurement of sulfite in various food and beverage samples (for example wine, dairy, canned products, juices, dried fruit, etc.).

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.

Component	Amount
Sulfite Assay Buffer Catalog Number MAK366A	25 mL
Sulfite Stabilizer Catalog Number MAK366B	25 mL
Sulfite Probe Catalog Number MAK366C	0.2 mL
Sulfite Oxidizing Mix Catalog Number MAK366D	80 μL
Sulfite Enzyme Mix Catalog Number MAK366E	1 vial
Sulfite Standard Catalog Number MAK366F	5 × 1 vial

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96 well plates
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Poly(vinylpolypyrrolidone) (PVPP) (Catalog Number 77627)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions.

Reagent Preparation

Sulfite Assay Buffer and Sulfite Stabilizer: Warm to room temperature prior to use.

Sulfite Probe: Light sensitive. Warm to room temperature, protected from light, prior to use.

Sulfite Oxidizing Mix: Light sensitive. Aliquot and store at –20 °C. Freeze/thaw cycles should be limited to two. Keep on ice, protected from light, during use.

Sulfite Enzyme Mix: Reconstitute with 220 μL of Sulfite Assay Buffer. Aliquot and store at –20 °C. Do not re-freeze aliquot once used. Keep on ice during use.

Sulfite Standard: Reconstitute each vial with 100 μL of Sulfite Stabilizer to generate a 2 M Sulfite Standard solution. Mix well and dissolve completely. The reconstituted standard is stable for up to 8 hours. Discard any unused standard.

Procedure

Sample Preparation

Wine

Sulfite concentration varies over a wide range depending on the sample. Red wines usually contain less sulfite concentration when compared to white wine. Sulfite concentrations range at 0-350 mg/L in red, pink, and white wine. Neutralization of red wine with NaOH may produce a normal change of color (blue). If strong color is observed, dilute appropriately.

1. White wine: Dilute samples with Sulfite Assay Buffer. Mix well and use for the assay directly. Add 1–50 μL into desired well(s) in a 96 well plate. Adjust the volume to 50 μL/well with Sulfite Assay Buffer.
2. Red wine: Neutralize samples (pH ~7) with 2 M NaOH (i.e., Add 35 μL of 2 M NaOH slowly to 1 mL of red wine). Mix well. Clarify sample by adding 50 mg of PVPP to the sample and vortex vigorously for 30 seconds. Centrifuge samples at 10,000 × *g* for 10 minutes at 4 °C. Collect the supernatant. Add 1–50 μL into desired well(s) in a 96 well plate. Adjust the volume to 50 μL /well with Sulfite Assay Buffer.
3. Recommended Dilution Factor:
   White Wine (10 to 50-fold)
   Red/Pink Wine (2 to 5-fold)

Food

Metabolites found in food samples may significantly inhibit the signal. Therefore, dilute the samples with Sulfite Assay Buffer. If interference is observed in the diluted samples as well, prepare parallel sample well(s) as sample background control(s) and make up the volume to 50 μL/well with Sulfite Assay Buffer.

Tissue Lysate and Biological Fluids

Tissue lysate, biological fluids, and any other sample with a high protein content should be deproteinized prior to use. Add sample to a Corning Spin-X UF concentrator, centrifuge at 10,000 × *g* for 10 minutes at 4 °C. Collect the filtrate

Note: For unknown samples, perform a pilot experiment and test several doses to ensure the readings are within the Standard Curve range. To ensure accurate determination of sulfite in the test samples or for samples having low concentrations of sulfite, spiking samples with a known amount of Sulfite standard (6 nmol) is recommended.

Standard Curve Preparation

Note: Prepare fresh diluted standard stock solutions and use within 2 hours.

1. Prepare a 10 mM Sulfite Standard by adding 5 μL of 2 M Sulfite Standard to 995 μL of Sulfite Stabilizer.
2. Further dilute to 1 mM Sulfite Standard by adding 100 μL of 10 mM Sulfite Standard to 900 μL Sulfite Stabilizer.
3. Prepare Sulfite Standards in desired wells of a clear flat-bottom 96 well plate according to Table 1.

Table 1.

Preparation of Sulfite Standards

Well	1 mM Premix	Sulfite Assay Buffer	Sulfite (nmol/well)
1	0 μL	50 μL	0
2	2 μL	48 μL	2
3	4 μL	46 μL	4
4	6 μL	44 μL	6
5	8 μL	42 μL	8
6	10 μL	40 μL	10

Oxidation Mix

1. Dilute Sulfite Oxidizing Mix 13-fold (i.e., 3 μL of Sulfite Oxidizing Mix plus 37 μL of Sulfite Assay Buffer). Mix enough reagents for the total number of wells to be assayed. For each well, prepare 10 μL of the diluted mix.
2. Add 10 μL of diluted Oxidation mix to standard and sample wells.
3. Incubate for 30 minutes at 37 °C.
4. Add 10 μL of Sulfite Assay Buffer to sample background control well(s).

Reaction Mix

Mix enough reagents for the total number of wells to be assayed including standards, samples, and background control(s). For each well, prepare 40 μL of Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mix

Reagent	Reaction Mix
Sulfite Assay Buffer	36 μL
Sulfite Enzyme Mix	2 μL
Sulfite Probe	2 μL

Mix well. Add 40 μL of the Reaction Mix to each well containing standards, samples, and background control(s). Mix well.

Measurement

Incubate the plate at 37 °C for 20 minutes protected from light. Measure the absorbance at 570 nm (A_{570}) in end-point mode.

Results

1. Subtract the 0 Sulfite Standard reading from all readings.
2. Plot the Sulfite Standard Curve.
3. If sample background control is significant, subtract sample background control reading from all sample readings.
4. Apply corrected A_{570} to Standard Curve to get nmol of Sulfite (B) in the sample well.

Total Sulfite Concentration (nmol/μL or mM) =

$$(B/V) \times D$$

where:
B = the amount of Sulfite in the sample well from the Standard Curve (nmol)
V = the volume of sample added to the reaction well (μL)
D = Sample dilution factor

Note: For spiked samples, correct for any sample interference by using the following equation:

Sulfite amount in spiked sample well (B) =

$$\frac{A_{570}\ Sample_{corrected}}{(A_{570}\ Spiked\ Sample_{corrected}) - (A_{570}\ Sample_{corrected})} * Sulfite\ spike\ (nmol)$$

Figure 1.
Typical Sulfite Standard Curve (0–10 nmol)

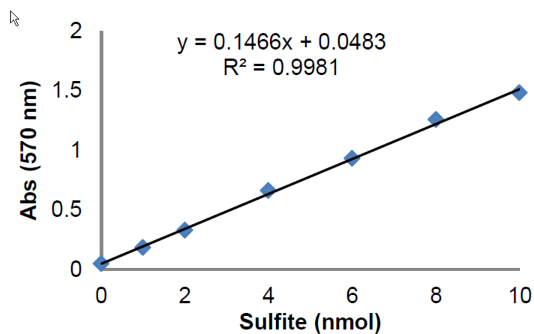
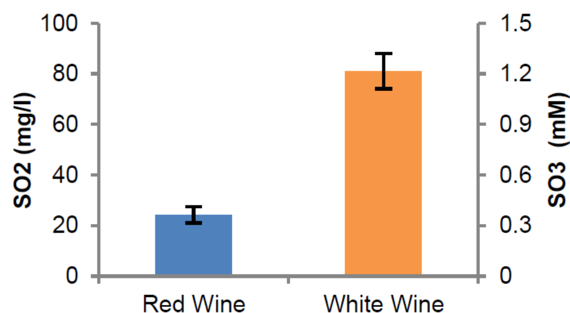


Figure 2.
Estimation of Total Sulfite Concentration in Two Wine Samples.



White wine was diluted using Sulfite Assay Buffer (16-fold) and 25 μL of diluted sample was spiked with 6 nmol of Sulfite Standard. Red wine was neutralized, clarified using PVPP, and spiked (6 nmol Sulfite) according to the procedure. Samples were then assayed following the kit procedure. Total Sulfite Concentrations (expressed as mg/L SO_2): Red Wine: 24.2, White wine: 80.9.

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