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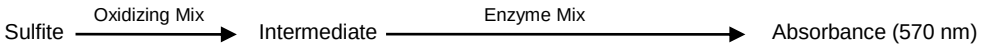
Total Sulfite Assay Kit (Colorimetric)

8/17

(Catalog # K699-100; 100 assays; Store at -20°C)

I. Introduction:

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 1000 mg/day. Sulfites are often considered as allergens due to adverse symptoms observed in asthmatic patients. Exogenous sources of sulphites include polluted air, food and beverages containing sulphur 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 cross-linking. 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. BioVision's 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, 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.



II. Application:

- Estimation of sulfite in various food and beverage samples.

III. Sample Type:

- Wine, dairy, canned products, juices, dried fruit, etc.

IV. Kit Contents:

Components	K699-100	Cap Code	Part Number
Sulphite Assay Buffer	25 ml	WM	K699-100-1
Sulphite Stabilizer	25 ml	NM	K699-100-2
Sulphite Probe	0.2 ml	Red	K699-100-3
Sulphite Oxidizing Mix	80 µl	Blue	K699-100-4
Sulphite Enzyme Mix	1 vial	Green	K699-100-5
Sulphite Standard	5 vials	Yellow	K699-100-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- 10 kDa Spin Column (Cat. # 1997)
- Multi-well spectrophotometer
- Polyvinylpolypyrrolidone (PVPP)

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read the entire protocol before performing the assay.

- **Sulphite Assay Buffer and Sulphite Stabilizer**: Store at -20°C. Bring to room temperature (RT) before use.
- **Sulphite Probe:** Light sensitive. Store at -20°C. Bring to RT before use.
- **Sulphite Oxidizing Mix:** Light sensitive. Aliquot and store at -20 °C. Freeze/thaw should be limited to two times. Keep on ice during use.
- **Sulphite Enzyme Mix:** Reconstitute with 220 µl of Sulphite Assay Buffer. Aliquot and store at -20°C. Freeze/thaw should be limited to one time. Keep on ice during use.
- **Sulphite Standard:** Reconstitute each vial with 100 µl of Sulphite Stabilizer to generate 2 M Sulfite Standard. Mix well. Dissolve completely. This reconstituted standard is stable for up to 8 hours. Discard unused standard.

VII. Sulfite Assay Protocol:

1. **Sample Preparation:** White wine: Dilute samples with Sulphite Assay Buffer. Mix well and use for the assay directly. Red wine: Neutralize samples (pH ~ 7) with 2 M NaOH (i.e. Add 35 µl of 2 M NaOH slowly to 1 ml red wine). Mix well. Clarify sample by adding 50 mg PVPP to the sample and vortex vigorously for 30 seconds. Centrifuge samples at 10,000 X g for 10 min. 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 Sulphite Assay Buffer.

   Notes:

   a. Metabolites found in food samples may significantly inhibit the signal. Therefore, it is recommended to dilute the samples with Sulphite 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 Sulphite Assay Buffer.

   b. Recommended Dilution Factor: White Wine (10-50), Red/Pink Wine (2-5). For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

c. 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.

d. For samples having high protein content (tissue lysate or biological fluids), we recommend sample deproteinization using our 10K Spin Columns **(Cat #1997)**. Add sample to the spin column, centrifuge at 10,000 X g, 10 min. at 4°C. Collect the filtrate.

e. To ensure accurate determination of sulfite in the test samples or for samples having low concentrations of Sulphite, we recommend spiking samples with a known amount of Sulfite standard (6 nmol)

2. **Standard Curve Preparation:** Prepare 10 mM Sulfite Standard by adding 5 µl of 2 M Sulphite Standard to 995 µl of Sulphite Stabilizer. Further dilute to 1 mM Sulfite Standard by adding 100 µl of 10 mM Sulfite Standard to 900 µl Sulphite Stabilizer. Add 0, 2, 4, 6, 8, and 10 µl of 1 mM Sulfite Standard into a series of wells in a 96-well clear plate to generate 0, 2, 4, 6, 8 and 10 nmol of sulfite/well. Adjust the volume to 50 µl/well with Sulphite Assay Buffer.

   Notes: Use diluted Sulfite standards (10 and 1 mM) within 2 hours. Prepare fresh diluted stocks prior any experiments.

3. **Oxidation Mix:** Dilute Sulphite Oxidizing Mix 13-fold (i.e. 3 µl Sulphite Oxidizing Mix + 37 µl Sulphite Assay Buffer). Mix enough reagents for the total number of wells to be assayed. For each well, prepare 10 µl of the diluted mix. Add 10 µl of diluted Oxidation mix to standard & sample wells. Incubate for 30 minutes at 37 °C. Add 10 µl Sulphite Assay Buffer to sample background control well(s).

4. **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 containing:

	Reaction Mix
Sulphite Assay Buffer	36 µl
Sulphite Enzyme Mix	2 µl
Sulphite Probe	2 µl

Mix well. Add 40 µl of the reaction Mix to each well containing standards, samples and Background Control(s). Mix well.

5. **Measurement:** Incubate the plate at 37°C for 20 min., protected from light. Measure the absorbance at 570 nm in an end point mode.

6. **Calculation:** Subtract 0 Sulphite Standard reading from all readings. Plot the Sulphite Standard Curve. If sample background control is significant, subtract sample background control reading from all sample readings. Apply corrected OD to Standard Curve to get B nmol Sulfite in the sample well.

Total Sulfite Concentration (C) = B/V X D nmol/µl or mM

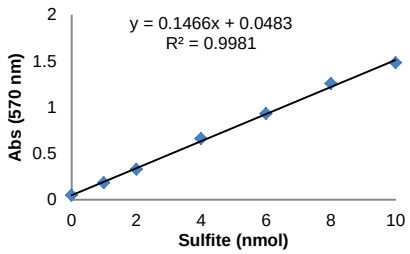
Where: **B** is amount of Sulfite in the sample well from Standard Curve (nmol)
V is sample volume added into the reaction well (µl)
D is sample dilution factor

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

$$\textbf{Sulfite amount in spiked sample well (B)} = \frac{\mathrm{OD}_{\text{sample (corrected)}}}{(\mathrm{OD}_{\text{sample+SUL Std (corrected)}})-(\mathrm{OD}_{\text{sample (corrected)}})} * \text{Sulfite spike (nmol)}$$

SO_2 molecular weight: 64 g/mol
SO_3^{2+}: 84 g/mol
100 mg/L SO_2 ≡ 1.56 mM SO_2

(a)



(b)

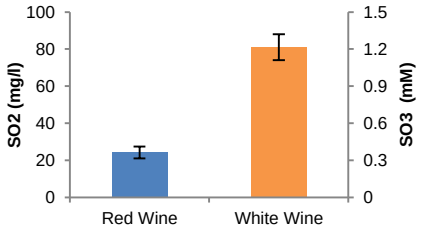


Figure: (a) Sulfite Standard Curve (0-10 nmol). **(b)** Estimation of Total Sulfite Concentration in two wine samples. White wine was diluted using SUL Assay Buffer (16-fold) and 25 µl of diluted sample was spiked with 6 nmol of Sulfite Standard. Red wine was neutralized, clarified using PVPP, and spiked (6 nmol Sulfite) according to the protocol. Samples were assayed following the kit protocol. Total Sulfite Concentrations (expressed as mg/l SO_2): Red Wine: 24.2, White wine: 80.9.

VIII. Related Products:

Malate Colorimetric Assay Kit (K637)
PicoProbe™ Fructose Fluorometric Assay Kit (K611)
Ammonia Colorimetric Assay Kit II (K470)
Urea Colorimetric Assay Kit II (K376)
Fructose Colorimetric/Fluorometric Assay Kit (K619)
Ammonia Colorimetric Assay Kit (K370)
Urea Colorimetric Assay Kit (K375)
D-Gluconate (D-Gluconic Acid) Assay Kit (Colorimetric) (K683)

FOR RESEARCH USE ONLY! Not to be used on humans.