TOTAL SULFITE
(LIQUID READY™)

PRODUCT INSTRUCTIONS

SKU: 700007891
K-TSULQR

08/26

(50 Manual Assays per Kit) or
(500 Auto-Analyzer Assays per Kit)

Megazyme® by NEOGEN®

Neogen Corporation, 620 Lesher Place, Lansing, MI 48912 USA | neogen.com
© Neogen Corporation 2026. All rights reserved. Neogen and Megazyme are registered trademarks, and Liquid Ready and Mega-Calc are trademarks of Neogen Corporation. All other marks mentioned are the property of their respective owners.

INTRODUCTION:

Total sulfite (TSO_2) refers to the combined free and bound sulfur dioxide (SO_2) in wine and food. In winemaking, sulfites are essential for microbial control, oxidation prevention, and color stability, particularly after malolactic fermentation and during aging. Once SO_2 binds to pigments and other compounds, it loses its preservative effect, making accurate measurement of total sulfite critical. Legal limits apply to TSO_2, not just free SO_2, so monitoring ensures compliance, product quality, and consumer safety. Regulatory bodies such as the EU and FDA require labelling above 10 mg/L, with wines typically restricted to 150–350 mg/L. Growing awareness of sulfite intolerance further reinforces the need for precise determination in wines, beverages, and foods.

PRINCIPLE:

The Total Sulfite assay is based on the oxidation of sulfite to sulphate under controlled conditions, followed by a colorimetric reaction with Ellman's reagent (DTNB). Ellman's reagent reacts with thiol groups generated during the process to produce a yellow chromophore (TNB^-), which is measured spectrophotometrically. The increase in absorbance at 400 nm is directly proportional to the total sulfite concentration.

$$\text{Total Sulfites} + \text{Ellman's Reagent (DTNB)} \rightarrow TNB^- + \text{oxidized sulfur species}$$

SPECIFICITY, SENSITIVITY AND LINEARITY:

- The assay is specific for Total Sulfites (SO_2 derived species).
- The limit of detection (LOD) is 0.002 g/L, and the limit of quantification (LOQ) is 0.005 g/L (using a sample volume of 0.1 mL).
- The recommended measuring range is between 0.005 g/L and 0.3 g/L (using a sample volume of 0.1 mL). This corresponds to 0.5 - 30 µg of Total Sulfites per assay.

INTERFERENCE:

Calcium Chloride interferes at concentrations above 5 g/L. L-Glutathione interferes at concentrations above 0.3 g/L. L-cysteine interferes at all tested concentrations. Other compounds containing free thiol groups may also cause interference. It is recommended that samples containing these interfering agents are diluted prior to testing.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. After use, the reagents may be disposed of with laboratory waste, in accordance with local regulations and guidelines.

NOTE: For more information regarding the performance of this product please refer to the associated validation report available from the Megazyme website. For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KIT CONTENTS:

Kits are designed for use in both manual and automated workflows. The reagents are sufficient for performing 50 assays in manual format or 500 assays in auto-analyzer format. The kit contains:

Reagent 1 (2 x 50 mL): Buffer
Contains sodium azide (0.02% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual labels for expiry date.

Reagent 2 (2 x 12.5 mL): Chromogen (DTNB)
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual labels for expiry date.

Calibrator (5 mL): Sulfites (0.3 g/L)
Ready to use.
Store at 4°C. See individual labels for expiry date.

PREPARATION OF REAGENT SOLUTIONS:

Bring all reagents to room temperature (20 - 25 °C) before use.

MANUAL ASSAY PROCEDURE:

Wavelength: 400 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 20 - 37°C
Final volume: 2.60 mL
Sample solution: 0.005 g/L to 0.3 g/L (i.e. 0.5 - 30 µg of Total Sulfite per cuvette)
Read against air (without a cuvette in the light path) or against water

Pipette into Cuvettes	Blank	Calibrator	Sample
Reagent 1	2.0 mL	2.0 mL	2.0 mL
Calibrator	-	0.1 mL	-
Sample	-	-	0.1 mL
Distilled Water	0.1 mL	-	-
Mix*, incubate for ~ 3 minutes at 20 - 37°C, then read the absorbances (A_1). Add Reagent 2 as described below:			
Reagent 2	0.5 mL	0.5 mL	0.5 mL
Mix*, incubate for ~ 3 minutes at 20 -37°C, then read the absorbances (A_2). **			

* Either by aspiration with the pipette tip used to dispense the liquid or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

** It may be necessary to check if the reaction has reached completion by continuing to read the absorbances at 1 minute intervals. If the reaction has not reached completion, continue to measure absorbances until the values measured either remain the same, or increase constantly over 1 minute. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of Reagent 2.

NOTE: For each batch of samples that are applied to the determination of TSO_2, a single point standard must be analyzed using the provided calibrator concurrently using the same batch of reagents. The reagent blank value must be determined once for each run and subtracted from each sample result.

CALCULATION:

NOTE: These calculations can be simplified by using the *Mega-Calc*™ tool, downloadable from the product page.

1. Calculation of the dilution factor (df)

Determine the dilution factor (df) based on the component ratios:

$$df = \frac{\text{Sample volume [mL] + R1 volume [mL]}}{\text{Total reaction volume [mL]}}$$

It follows for the Total Sulfite manual assay procedure:

$$df = \frac{0.1 + 2.0}{2.6} = 0.808$$

2. Calculation of the absorbance difference $\Delta A_{Sulfite}$

$$\Delta A_{Sulfite} = (A_2 - df \times A_1)_{sample/calibrator} - (A_2 - df \times A_1)_{blank}$$

It follows for the Total Sulfite manual assay procedure:

$$\Delta A_{Sulfite} = (A_2 - 0.808 \times A_1)_{sample/calibrator} - (A_2 - 0.808 \times A_1)_{blank}$$

NOTE: Increasing or decreasing the sample volume with unchanged reagent volumes requires recalculation of the dilution factor; if volumes are changed, the system and performance may be affected.

3. Calculation of the Total Sulfite content

The concentration of total sulfites can be calculated as follows:

$$c = \frac{\Delta A_{Sample}}{\Delta A_{Calibrator}} \times C_{Calibrator} \quad [g/L]$$

Since the concentration of the calibrator is 0.3 g/L, this gives the following calculation formula:

$$c = \frac{\Delta A_{Sample}}{\Delta A_{Calibrator}} \times 0.3 \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the sample dilution factor, F.

4. Calculation of the Total Sulfite content in solid or semi-solid samples:

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the content g/100 g and mg/kg is calculated from the amount weighed as follows:

$$\frac{C_{Sulfite}\ [g/L\ sample\ solution]}{weight_{sample}\ [g/L\ \ sample\ solution]} \times 100 \quad [g/100g]$$

$$C_{Sulfite}\ [g/100g] \times 10{,}000 \quad [mg/kg]$$

AUTO-ANALYZER ASSAY PROCEDURE:

This kit has been designed for auto-analyzers and can be adapted to most instruments. A sample method is shown below (validated on the Awareness ChemWell®-T analyzer).

NOTE: For each batch of samples that is applied to the determination of Total Sulfite a calibration curve must be created concurrently using the same batch of reagents.

Parameter	Details
Wavelength	400/630 nm (primary)
Temperature	20 - 37°C
Test	End-point test with following test sequence: – Add Reagent 1 **[0.2 mL]** – Add Sample or Calibrator **[0.01 mL]** – Pre-incubate 1 - 3 minutes [20 - 37°C] – Measure A_1 at 400/630 nm – Add Reagent 2 **[0.05 mL]** – Incubate 3 minutes at [20 - 37°C] – Measure A_2 at 400/630 nm – Calculate $A_2 - A_1$ against calibration curve
Calibration	Calibrate using 2 – 4 calibrators ranging from 0 – 0.3 g/L. The calibration curve is linear. An example of how to use the standard supplied with the kit to create a calibration curve is shown below: Calibrator 1 0 g/L (use 0.1% citric acid solution) Calibrator 2 0.01 g/L (dilute Standard 30-fold) Calibrator 3 0.15 g/L (dilute Standard 2-fold) Calibrator 4 0.3 g/L (use Standard as-is) *Perform all dilutions in 0.1% citric acid.*

SAMPLE PREPARATION:

1. Sample dilution

The amount of Total Sulfite present in the sample should range from 0.005 g/L to 0.3 g/L. If the value for $\Delta A_{Sulfite}$ is too low (e.g. <0.1), weigh more sample or decrease the dilution. If the value for $\Delta A_{Sulfite}$ is too high (e.g. >3.0), increase the dilution in 0.1% citric acid.

Sample Dilution Table

Estimated Concentration of Total Sulfite (g/L)	Dilution with 0.1% citric acid	Sample Dilution factor (F)
< 0.3	No dilution required	1
0.3 – 3	1 mL sample + 9 mL 0.1% citric acid	10
3 -30	1 mL sample + 99 mL 0.1% citric acid	100

2. General sample preparation guide

IMPORTANT NOTE: Carrez precipitation must not be used for sue testing, and deproteinization with acids is not recommended. These procedures can cause loss or alteration of sulfite during sample preparation, leading to inaccurate results. Similarly, PVPP treatment must not be used for sulfite determination, as it removes phenolic and carbonyl compounds that participate in sulfite equilibria, resulting in shifts in free and bound sulfite levels and unreliable measurements. The below are suggested sample preparation examples only. If you have questions about these or other matrices, please contact your local sales representative for support

- Clear, slightly colored, and approximately neutral, liquid samples at concentrations up to 0.3 g/L can be used directly in the assay.
- Turbid samples should be filtered or centrifuged.
- Samples containing carbon dioxide should be degassed by gentle agitation or stirring with a glass rod.
- Solid samples should be homogenized, extracted in 0.1 % citric acid, and filtered or centrifuged if necessary.

3. Suggested sample preparation examples

(a) Determination of Total Sulfites in wine. Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of wine for 5 minutes at 15,000 g. *Typically, no dilution is required.*

(b) Determination of Total Sulfites in beer (e.g. lager): Remove carbonation by stirring a sample in a beaker for approximately 60 seconds using a glass rod. Pass through a 0.2 micron syringe filter and use the clear filtrate in the assay. *Typically, no dilution is required.*

(c) Determination of Total Sulfites in dried fruits (e.g. dried mango): Prepare the dried mango sample by homogenizing in a blender for 15 seconds. Add 10 g of the homogenized dried mango to a 100 mL graduated cylinder and adjust to 100 mL with 0.1% citric acid. Transfer the contents to a blender and homogenize the sample for 30 seconds. Centrifuge an aliquot for 5 minutes at 15,000 g. *Typically, no dilution is required*

(d) Determination of Total Sulfites in sugar (e.g. Brown Sugar): Add 50 g of sugar to a 100 mL volumetric flask and adjust to 100 mL with 0.1% citric acid. Transfer the contents to a beaker and stir the solution at room temperature until all the sugar is visibly dissolved (~10 minutes). *Typically, no dilution is required.*

(e) Determination of Total Sulfites in pickles: Empty the entire contents of the pickle sample (pickles and brine) into a blender. Blend until a homogenous slurry is obtained. Centrifuge an aliquot for 5 minutes at 15,000 g. Carefully remove 750 µL of the supernatant, add 250 µL of distilled water and mix thoroughly to obtain a sample with a dilution factor of 1.33. Analyze the diluted sample according to the assay procedure. *Note: Pickle samples should not be analyzed neat (undiluted) due to matrix interference, which may affect assay performance and result accuracy. A minimum dilution factor of 1.33 is required for analysis. If additional matrix interference is observed, further dilution of the sample may be necessary and can be increased as required.*

SERVICES AND TECHNICAL SUPPORT

Please reach out to your local sales representative should you require any assistance, particularly in relation to:

- Troubleshooting
- Data analysis
- Additional matrix testing
- Application support in relation to automated analyzers

Supporting documents can be found on the product page:

- Quick Reference Guide
- Mega-Calc™
- Safety Data Sheets (SDS)
- Certificates Of Analysis (COA)
- Validation Report

Contact us for more information: neogen.com/contact

Without guarantee

The information contained in this assay protocol is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents.

User Responsibility:

- Users are responsible for familiarizing themselves with product instructions and information. Visit our website at neogen.com, or contact your local Neogen® representative or authorized distributor for more information.
- When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, laboratory technique and the sample itself may influence results.
- It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and challenges to satisfy the user that the chosen test method meets the user's criteria.
- It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.
- As with any test method, results obtained do not constitute a guarantee of the quality of the matrices or processes tested.

Terms and Conditions:
Neogen's full terms and conditions are available online.