# FREE SULFITE
# (LIQUID READY™)

PRODUCT INSTRUCTIONS

## SKU: 700007892
## K-FSULQR

03/26

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

Megazyme® by NEOGEN®

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## INTRODUCTION:

Free sulfite ($FSO_2$) refers to the portion of sulfur dioxide ($SO_2$) that remains unbound and fully active in wine or food. Unlike bound $SO_2$, which is tied to sugars, pigments, or other compounds, free $SO_2$ provides the primary antimicrobial and antioxidant protection, critical for preventing spoilage, oxidation, and maintaining color stability. Its effectiveness depends on pH and temperature, making accurate measurement essential for quality control and shelf-life prediction. Regulatory compliance and consumer safety also hinge on monitoring free $SO_2$, as it determines the actual preservative strength of the product. While legal limits apply to total sulfite, understanding and controlling free $SO_2$ ensures optimal protection without exceeding thresholds, especially in response to growing concerns about sulfite sensitivity.

## PRINCIPLE:

The Free Sulfite assay is based on the reaction of sulfur dioxide ($SO_2$) with fuchsin dye and an aldehyde reagent under controlled acidic conditions. Free $SO_2$ reacts with the aldehyde to form a stable addition compound, while simultaneously decolorizing the fuchsin dye. This color change is measured spectrophotometrically and is directly proportional to the Free Sulfite concentration.

As the reaction between $FSO_2$ and the aldehyde proceeds, the equilibrium between "bound" $SO_2$ and free $SO_2$ shifts toward the free form, ensuring accurate determination of the active preservative fraction. In red wines, this equilibrium shift is linear, allowing precise quantification even in complex matrices.

$$\text{Free Sulfites } (FSO_2) + \text{Aldehyde} + \text{Fuchsin} \rightarrow \text{Leucofuchsin (colorless complex)}$$

## SPECIFICITY, SENSITIVITY AND LINEARITY:

- The assay is specific for Free Sulfites ($FSO_2$).
- The limit of detection (LOD) is 0.0003 g/L, and the limit of quantification (LOQ) is 0.001 g/L (using a sample volume of 0.1 mL).
- The recommended measuring range is between 0.003 g/L and 0.125 g/L (using a sample volume of 0.1 mL). This corresponds to 0.3 – 12.5 µg of Free Sulfites per assay.

## INTERFERENCE:

Ascorbic Acid interferes at concentrations above 2.5 g/L. It is recommended that samples with stated contents of this interfering agent 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):** Chromogen (Basic Fuchsin)
Ready to use.
**Store at 4°C. See individual labels for expiry date.**

**Reagent 2 (2 x 12.5 mL):** Buffer
Ready to use.
**Store at 4°C. See individual labels for expiry date.**

**Calibrator (5 mL):** Sulfite (0.125 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:** 575 nm
**Cuvette:** 1 cm light path (glass or plastic)
**Temperature:** 20 - 37°C
**Final volume:** 2.60 mL
**Sample solution:** 0.003 g/L to 0.125 g/L (i.e. 0.3 – 12.5 µg of Free 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®.

****IMPORTANT NOTE:** For **red wines**, the A2 absorbance reading must be taken exactly 3 minutes (±30 s) after addition of Reagent 2 to prevent inclusion of time-dependent drift.

**NOTE:** For each batch of samples that are applied to the determination of Free Sulfite, a single point standard must be performed with 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:

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

It follows for the Free 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 Free 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 Free Sulfite content

The concentration of Free Sulfite 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 0.125 g/L, this gives the following calculation formula:

$$c = \frac{\Delta A_{Sample}}{\Delta A_{Calibrator}} \times 0.125 \quad [g/L]$$

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

**4. Calculation of the Free 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) is calculated from the amount weighed as follows:

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

## 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 Free Sulfite a calibration curve must be created concurrently using the same batch of reagents.

| Parameter | Details |
|---|---|
| Wavelength | 575/630 nm (primary) |
| Temperature | 20 - 37°C |
| Test | End-point test with following test sequence:<br>– Add Reagent 1 **[0.2 mL]**<br>– Add Sample or Calibrator **[0.01 mL]**<br>– Pre-incubate 1 - 3 minutes [20 - 37°C]<br>– Measure $A_1$ at 575/630 nm<br>– Add Reagent 2 **[0.05 mL]**<br>– Incubate 3 minutes at [20 - 37°C]<br>– Measure $A_2$ at 575/630 nm<br>– Incubate 3 minutes [20 - 37°C]<br>– Measure A3 at 575/630 nm<br>– Calculate ($A_2 – A_1$) against calibration curve |
| Calibration | Calibrate using 2 – 4 calibrators ranging from 0 – 0.125 g/L.<br>The calibration curve is linear.<br><br>An example of how to use the standard supplied with the kit to create a calibration curve is shown below:<br>Calibrator 1 0 g/L (use 0.1% citric acid)<br>Calibrator 2 0.00625 g/L (dilute Standard 20-fold)<br>Calibrator 3 0.0625 g/L (dilute Standard 2-fold)<br>Calibrator 4 0.125 g/L (use Standard as-is)<br>*Perform all dilutions with 0.1% citric acid.* |

## SAMPLE PREPARATION:

### 1. Sample dilution

The amount of Free Sulfite present in the sample should range from 0.003 g/L to 0.125 g/L. If the value of $\Delta A_{Sulfite}$ is too low (e.g. <0.1), weigh more sample or decrease the dilution. If the value $\Delta A_{Sulfite}$ is too high (e.g. >3.0), increase the dilution in distilled water.

**Sample Dilution Table**

| Estimated Concentration of Free Sulfite (g/L) | Dilution with 0.1 % citric acid | Sample Dilution factor (F) |
|---|---|---|
| < 0.125 | No dilution required | 1 |
| 0.125 – 1.25 | 1 mL sample + 9 mL 0.1% citric acid | 10 |
| 1.25 -12.5 | 1 mL sample + 99 mL 0.1% citric acid | 100 |

### 2. General sample preparation guide

**IMPORTANT NOTE:** Carrez precipitation must not be used for sulfites testing, and deproteinization with acids is not recommended. These procedures can cause loss or alteration of sulfites during sample preparation, leading to inaccurate results. Similarly, PVPP treatment must not be used for sulfites determination, as it removes phenolic and carbonyl compounds that participate in sulfites equilibrium, resulting in shifts in free and bound sulfite levels and unreliable measurements. For reliable measurement, follow the specified protocol without additional precipitation, clarification, or acid based treatment steps. 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 a concentration up to 0.125 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 Free Sulfite 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 Free Sulfite 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.*

## 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

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- 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.

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