TOTAL SULFITE
(LIQUID READY™)

VALIDATION REPORT

SKU: 700007891
K-TSULQR

11/26

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INTRODUCTION:

The Total Sulfite Assay Kit (Liquid Ready) is designed to be a robust, quick and easy method for the measurement of Total Sulfite in various matrices and is fully automatable for high throughput analysis of samples. Data presented in this report validates that this method is fit for the purpose intended.

RECOMMENDATIONS FOR ANALYSIS:

Please reach out to your local sales representative or to the technical team should you require any assistance, particularly in relation to assay troubleshooting, data analysis, additional matrix testing and application support in relation to automated analyzers.

- This test should only be carried out by trained laboratory employees. The product instructions must be followed to help ensure an accurate and robust result.
- Store the kit at 2 - 8 °C.
- Ensure all kit components come to room temperature 20 - 25 °C before use.
- Use the contents of bottles 1, 2 & 3 as supplied.
- Use of repetitive pipettor is recommended to reduce the risk of pipetting error.
- The reagent blank value must be determined once for each set of determinations and subtracted from each sample result.
- The calibrator value must be determined for each set of determinations and used to calculate the Total Sulfite content of each sample result.
- Users should perform matrix validation experiments prior to routine use. This process will highlight any problematic matrices encountered.
- Use separate pipette tips for each sample extract and control solutions to reduce the risk of cross-contamination. Additionally, pre-flush the tip before pipetting.
- When testing solid samples, ensure a representative portion is homogenized before weighing.

EQUIPMENT (RECOMMENDED):

1. Positive displacement pipettor (e.g. Eppendorf Multipette® with Combitip®) or micro-pipettors (e.g. Gilson® Pipetman®).
2. Macro-cuvettes (e.g. BRAND® PMMA, 1 cm pathlength).
3. Cuvette stoppers/caps or sealing film (e.g. Parafilm™).
4. Heat block incubator (or equivalent) for cuvettes (not required if ambient temperature is in the range of 20-37°C).
5. Spectrophotometer capable of reading at 400 nm.
6. Syringe filters (0.2 micron, e.g. AGILENT® Nylon or equivalent) or Filter paper (e.g. Sartorius® grade 292 or equivalent).
7. Beaker (e.g. BRAND 100 mL capacity).
8. Glass rod (e.g. Aldrich® stirring rods).
9. Glass Test Tubes (e.g. Pyrex® 16 mm x 100 mm).
10. Blender (e.g. Nutribullet® or equivalent).
11. Wide-mouth bottle (e.g. Duran® bottles, 100 mL).
12. Refrigerator or ice bath.
13. 0.1% citric acid solution

SUMMARY OF PERFORMANCE DATA:

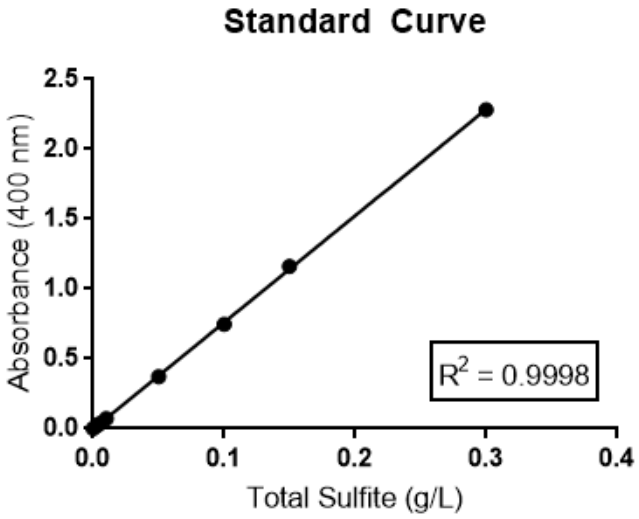
All testing was performed using the standard manual assay described in the product instructions. Results are summarized in the table below:

Recommended working range	0.005 g/L – 0.3 g/L
Limit of Detection (LOD)	0.002 g/L
Limit of Quantification (LOQ)	0.005 g/L
Limit of Precision	0.005 g/L
Specificity	Sulfur Dioxide
Bias %	- 1.5 % to 2.23%
Acceptable Recovery of Standards	95 % - 105 %
Stability Studies	24 months shelf life from date of manufacture, see product label for expiry
Robustness	Reaction takes 3 mins to reach completion at 20^{o}C, 25^{o}C and 37^{o}C
	Full recovery after 3 freeze-thaw cycles
Verified Matrices	Red Wine, White Wine, Beer, Brown Sugar, Shrimp and Dried Mango
Ruggedness	CV < 5 % for aqueous sulfite samples
	CV < 10 % for the range of matrices tested (red wine, white wine, beer, brown sugar and dried mango)
Cross-reactivity	Calcium chloride > 5 g/L. L-glutathione > 0.025 g/L. Other Thiol-containing compounds, such as L-cysteine, react directly within the assay system and may cause an overestimation of sulfite concentrations.
Selectivity	Recovery between 95 % - 105 % for sodium sulfite, sodium bisulfite, sodium metabisulfite, and potassium disulfite
Matrix Interference	Recovery between 95 % - 105 % in red wine, white wine, beer, brown sugar and dried mango.

LINEARITY AND WORKING RANGE:

The recommended measurement range for Total Sulfite is 0.005 g/L to 0.300 g/L. Samples containing analyte concentrations above this range should be appropriately diluted with 0.1% citric acid prior to analysis to ensure accurate quantification. Samples containing less than 0.005 g/L Total Sulfite will fall outside the validated range and will not meet the acceptance criteria.

Total Sulfite (g/L)	$\Delta_{absorbance}$ (400 nm)	Recovery (%)
0.000	0.000	-
0.003	0.021	92.276
0.005	0.037	96.852
0.010	0.072	95.280
0.050	0.372	97.414
0.100	0.746	97.752
0.150	1.162	101.528
0.300	2.283	100.177



LIMIT OF DETECTION, QUANTIFICATION AND PRECISION:

The LOD is the lowest concentration of the analyte that can be detected by the method. This was determined by testing 21 replicates of the blank (i.e. adding 100 µL of water instead of sample). The $\Delta A_{Limit\ of\ Detection}$ is calculated as 3.3 x s'0; where s'0 is the standard deviation of the blank $\Delta_{Absorbance}$ reading.

The LOQ is the lowest level at which the kit's performance is acceptably repeatable. This was determined by testing 20 replicates of the blank (i.e. adding 100 µL of water instead of sample). The $\Delta A_{Limit\ of\ Quantification}$ is calculated as kQ x s'0; where s'0 is the standard deviation of the blank $\Delta_{Absorbance}$ reading. The IUPAC default value for kQ is 10.

The Limit of Precision refers to the lowest analyte concentration at which acceptable recoveries (±5%) are consistently achieved under routine conditions. This parameter was determined experimentally by analyzing increasing concentrations of the analyte, beginning at the limit of quantification (LOQ). The assessment focused on identifying the lowest concentration level that still met the predefined recovery criteria.

$Abs_{Limit\ of\ Detection}$	Limit of Detection (g/L)	$Abs_{Limit\ of\ Quantification}$	Limit of Quantification (g/L)	Precision Limit (g/L)
0.015	0.002	0.045	0.005	0.005

NOTE: The above detection limits were calculated based on assay concentration (i.e. samples post-extraction). The dilution used in pre-treatment must be accounted for when establishing the detection limits for specific samples.

TRUENESS AND *BIAS:*

The trueness of the Total Sulfite Kit (Liquid Ready) was evaluated using validated aqueous standards at 0.005 g/L; 0.150 g/L and 0.300 g/L. Trueness was assessed by comparing the mean result (X), obtained through the standard manual protocol, with a suitable reference material.

Relative Bias is calculated in percent as: $b(\%) = (X - X_{ref}) / X_{ref} \times 100$

Assay Temperature (°C)	Replicates, n	Concentration (g/L)	Standard Deviation	CV (%)	Recovery (%)	*Bias (%)*
20°C	10	0.005	0.0001	2.247	100.81	0.813
	10	0.153	0.0005	0.306	102.23	2.230
	10	0.304	0.0013	0.425	101.36	1.358
25°C	10	0.005	0.0001	1.220	98.70	-1.303
	10	0.152	0.0013	0.849	101.35	1.346
	10	0.302	0.0014	0.474	100.77	0.767
37°C	10	0.005	0.0001	2.293	98.50	-1.496
	10	0.153	0.0004	0.252	101.68	1.675
	10	0.303	0.0011	0.364	101.093	1.093

The recovery of the sample solutions fell within the acceptance criteria of 100 ± 5 %, with a coefficient of variation (%CV) below 5 %. The calculated bias for the method ranged from -1.50 % to 2.23 %, indicating acceptable agreement with the expected values.

INTERFERENCE AND SELECTIVITY:

Selectivity Towards Sulfite Derivatives

Selectivity towards different sulfite forms was assessed using standards prepared from a range of sulfite-containing compounds such as commonly used inorganic sulfite salts, including sodium sulfite, sodium bisulfite, sodium metabisulphite, potassium disulfite and hydroxymethanesulfonate (HMS), a covalent addict of sulfite with formaldehyde prepared to 0.3 g/L.

Sulfite Form	Extraction buffer	Reaction Time (mins)	Concentration (g/L)	Recovery (%)
Sodium Sulfite	0.1% Citric Acid	3	0.295	98.461
Sodium Bisulfite	0.1% Citric Acid	3	0.314	104.716
Sodium Metabisulfite	0.1% Citric Acid	3	0.306	101.996
Potassium Disulfite	0.1% Citric Acid	3	0.291	96.450
HMS	0.1% Citric Acid	3	0.151	50.329
	0.1M NaOH + 1mM EDTA	3	0.274	91.225

The assay demonstrated equivalent response to commonly used inorganic sulfite salts, with recoveries of 96-105%. HMS showed strong assay response only when extracted under alkaline conditions (0.1 M NaOH with EDTA, confirming that this bound sulfite species requires specific treatment to ensure quantitative release and detection.

NOTE: Alkaline extraction might interfere with thiols present in complex matrices.

Cross Reactivity

The method was evaluated in the presence of potential interfering compounds. This was achieved by spiking a fixed concentration of each interfering agent into a validated aqueous standard containing a known concentration of Sulfite and assessing the recovery.

NOTE: Interfering substances in the sample being analyzed can be identified by including a certified reference material. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding sulfite to the sample in the initial extraction steps.

Interfering Agent	Concentration in assay (g/L)	Recovery (%)
D-Glucose	300	101.9
D-Fructose	300	101.7
D-Galactose	5	102.2
D-Mannose	5	101.0
Sucrose	200	100.9
Lactose	5	101.5
Glycerol	20	101.0
Sorbitol	10	100.9
Malic Acid	50	102.2
Tartaric Acid	10	102.6
Citric Acid	50	101.9
Succinic Acid	5	103.0
Galacturonic Acid	10	103.0
Acetic Acid	10	103.0
Ascorbic Acid	10	101.1
Sodium Chloride	10	103.3
Calcium Chloride	10	114.6
	5	101.5
Ethanol	120	101.6
L-Cysteine	0.5	210.9
	0.1	111.6
L-Glutathione	0.2	130.4
	0.025	104.7
Acetaldehyde	0.1	100.6
Taurine	1	100.5

* Red font indicates possible interference.

The concentrations of the interfering agents were selected to reflect levels likely to be encountered in relevant sample matrices. The data suggests possible cross-reactivity with three compounds: Calcium Chloride (>5 g/L), L-Cysteine (directly interferes as low as 0.1 g/L), and L-Glutathione (> 0.025 g/L). These compounds showed positive interference due to their reaction with DTNB, leading to overestimation of sulfite. Excluding these known interferents, all compounds tested met the recovery acceptance criterion of 100 ± 5% indicating that no significant cross-reactivity occurred with any of the other tested substances.

Matrix Interference

Matrix-related interference for this method was assessed by spiking extracted samples from complex matrices with a known concentration of a validated aqueous sulfite standard and measuring the recovery.

NOTE: All matrices were prepared according to the sample preparation methods described in the product instruction document which can be found on the product webpage.

Matrix	Spike Measured in Assay (g/L)	In-matrix Spike Recovery (g/L)	In-matrix Spike Recovery (%)
Red Wine	0.152	0.146	96.232
White Wine	0.149	0.149	100.291
Beer	0.148	0.150	101.548
Brown Sugar	0.150	0.149	99.402

Dried Mango	0.150	0.149	99.286
Mean	0.150	0.149	99.352
Standard deviation	0.0015	0.0015	1.965
CV (%)	0.990	1.021	1.977

A total of six matrices were evaluated, with all relevant samples demonstrating recoveries within the acceptable range of 95–105 %. Additionally, CV (%) were below 5%, indicating good method precision across different sample types.

ROBUSTNESS AND STABILITY

Storage Temperature

To evaluate the storage stability of the test kit components, all materials were stored at 4°C. Real-time performance and enzyme activity testing were conducted on a monthly basis. Slope-based trend analysis was employed to predict the shelf life of the product.

Storage Temperature	Reagent Tested	Stability Data
4°C	Reagent 1	24 months shelf life from date of manufacture, see product label for expiry
	Reagent 2	
	Calibrator	

The storage robustness of the kit was further evaluated through three freeze-thaw cycles. All kit components were frozen at -20°C overnight and allowed to thaw before testing. This process was repeated three times using the same reagents.

	Total Sulfite Recovery (g/L)			
Expected Sulfite (g/L)	T0	1st Cycle	2nd Cycle	3rd Cycle
0.005	0.005	0.005	0.005	0.005
0.150	0.150	0.153	0.150	0.151
0.300	0.300	0.301	0.300	0.296

Reagent performance remained stable across three freeze–thaw cycles at all concentrations tested. Recoveries in each cycle were consistent with the T0 values, and the single-factor ANOVA showed no statistically significant differences between cycles ($F = 0.0001$, $F_{crit} = 4.0662$, $p = 1.000$). These results demonstrate that the reagent is stable through at least three freeze–thaw cycles.

Assay Temperature

Chemical test kits can be sensitive to environmental conditions, with temperature being a key factor influencing reaction rate and analyte recovery. The Total Sulfite (Liquid Ready) was evaluated at three different reaction temperatures: 20°C, 25°C, and 37°C. Recovery rates and reaction times were analyzed across these conditions.

	Total Sulfite Recovery (g/L)		
Expected Total Sulfite (g/L)	20°C	25°C	37°C
0.005	0.005	0.005	0.005
0.150	0.148	0.150	0.150
0.300	0.296	0.300	0.301
Reaction Time	3 min	3 min	3 min

Recommended time	*3-minute reaction time is recommended*

Across the three sulfite concentrations tested (0.005, 0.150, and 0.300 g/L), recoveries obtained at 20 °C and 37 °C were highly consistent with the corresponding 25 °C measurements. Only minor numerical differences were observed between temperatures, and these did not indicate any systematic bias. Two-sample t-tests confirmed that temperature-related differences were not statistically significant. For the 20 °C vs 25 °C comparison, the t-statistic ($t = -1.45$) was well below the critical value ($T_{crit} = 4.30$), with a p-value of 0.285. Similarly, the 37 °C vs 25 °C comparison yielded a non-significant result ($t = -1.72$, $df = 2$, $p = 0.227$). In both cases, the null hypothesis of equal means could not be rejected. Overall, the results demonstrate that assay recovery is not temperature-dependent within the evaluated range. Recoveries at 20 °C and 37 °C can therefore be considered equivalent to those obtained at the reference temperature of 25 °C.

PRECISION AND REPEATABILITY

Precision

Precision reflects the consistency of results obtained under varying conditions, including different days, analysts, and reagent lots. The precision of the test kit was evaluated using verified aqueous standards.

Total Sulfite (g/L)	Replicates, n	Mean (g/L)	Standard Deviation	CV (%)
0.005	24	0.005	0.0001	2.13
0.150	24	0.153	0.0009	0.57
0.300	24	0.303	0.0035	1.17

At the low (0.005 g/L), mid (0.150 g/L), and high (0.300 g/L) sulfite levels (n = 24 per level), all recovery values fell within the acceptance range of 100 ± 5%, and %CV values were well below the 5% precision limit. These results demonstrate that the method is both precise and repeatable under typical laboratory conditions.

Matrix Repeatability

Matrix repeatability was assessed two analysts, on two lots, over one day, using three replicates per extraction for each matrix. Sample preparation was conducted daily to evaluate both the precision of analyte extraction and the repeatability of the assay method. All extractions followed the sample preparation protocol outlined in the product instructions.

Matrix	Replicates, n	Mean result (g/L)	Standard Deviation	CV (%)
Red Wine	12	0.040	0.003	7.594
White Wine	12	0.037	0.003	7.674
Beer	12	0.002	0.000	5.593
Dried Mango	12	0.099	0.006	6.314
Brown Sugar	12	0.001	0.000	10.909

Mean results for red wine, white wine, beer (lager) and dried mango fall within the validated working range and demonstrate acceptable variability across most matrices. Precision was acceptable for the majority of matrices, with % CV values below 10 %, including samples with concentrations below the LOQ (i.e. beer). The elevated % CV observed for brown sugar is attributed to results close to the LOD, where increased variability is expected. Overall, the data support the suitability of the method across the specified concentration range and matrices.

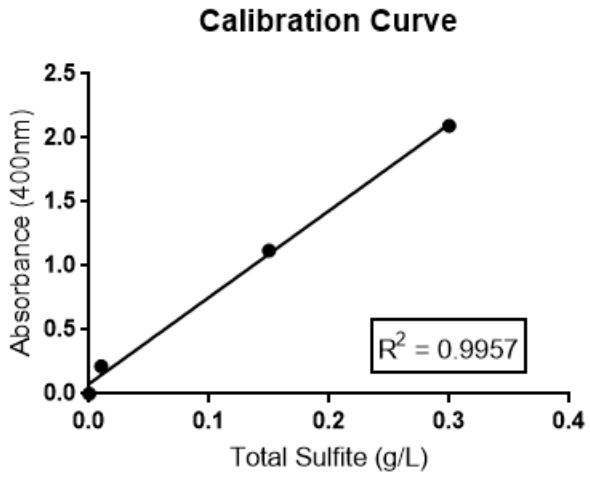
NOTE: Users should perform matrix validation work prior to routine use. This process will highlight any problematic matrices encountered. If you have questions about these or other matrices, please contact your local sales representative for support.

METHOD AUTOMATION

The Total Sulfite (Liquid Ready) Assay Kit is specifically designed for use with biochemistry analyzers and can be readily adapted to a wide range of instrumentation. Quantification of total sulfite is achieved through a single-test format using a linear calibration fit. The performance data presented below were generated using a ChemWell-T analyzer operating at 37°C.

NOTE: For each batch of samples that is applied to the determination of total sulfite a calibration curve must be performed concurrently using the same batch of reagents.

Total Sulfite (g/L)	Replicates, n	Mean result (g/L)	Standard Deviation	CV (%)
0.010	3	0.010	0.000	2.068
0.150	3	0.150	0.002	1.311
0.300	3	0.300	0.003	0.898



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 in the product page:

- Product Instructions
- Quick Reference Guide
- Mega-Calc™
- Safety data sheets (SDS)
- Certificate of analysis (CoA)

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.