FREE SULFITE
(LIQUID READY™)

VALIDATION REPORT

SKU: 700007892
K-FSULQR

11/26

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INTRODUCTION:

The Free Sulfite Assay Kit (Liquid Ready) is designed to be a robust, quick and easy method for the measurement of free 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 Free 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 575 nm.
6. Syringe filters (0.2 micron, e.g. AGILENT® Nylon or equivalent) or Filter paper (e.g. Sartorius® grade 292 or equivalent).
7. 0.1% Citric Acid Solution

SUMMARY OF PERFORMANCE DATA:

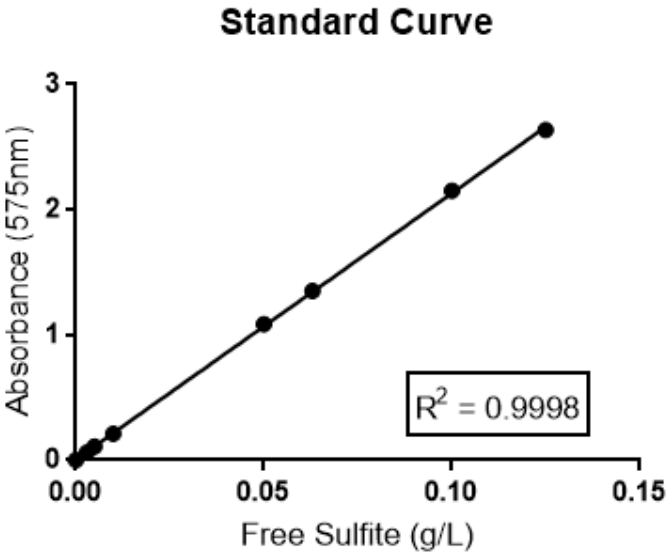
All testing was performed using the standard manual assay described in the product instructions. Results are summarized in the table below:

Recommended working range	0.003 g/L – 0.125 g/L
Limit of Detection (LOD)	0.0003 g/L
Limit of Quantification (LOQ)	0.001 g/L
Limit of Precision	0.003 g/L
Specificity	Unbound Sulfites
Bias %	- 2.24 % to 1.34 %
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, rose wine, beer
Ruggedness	CV < 5 % for aqueous sulfite samples
	CV < 10 % for the range of matrices tested (Red wine, white wine, rose wine and beer)
Cross-reactivity	Ascorbic Acid interferes at concentrations above 2.5 g/L.
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, rose wine and beer.

LINEARITY AND WORKING RANGE:

The recommended measurement range is 0.003 g/L to 0.125 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 0.003 g/L Free Sulfite will fall outside the validated range and will not meet the acceptance criteria.

Free Sulfite (g/L)	$\Delta_{absorbance}$ (575 nm)	Recovery (%)
0.000	0.000	-
0.003	0.062	96.585
0.005	0.109	102.505
0.010	0.212	99.206
0.050	1.085	101.430
0.063	1.352	101.492
0.100	2.152	100.547
0.125	2.635	98.903



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.

$\Delta A_{Limit\ of\ Detection}$	Limit of Detection (g/L)	$\Delta A_{Limit\ of\ Quantification}$	Limit of Quantification (g/L)	Precision Limit (g/L)
0.0052	0.0003	0.0156	0.0010	0.0030

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 Free Sulfite Assay Kit (Liquid Ready) was evaluated using validated aqueous standards at 0.003 g/L; 0.063 g/L and 0.125 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 (^oC)	Replicates, n	Concentration (g/L)	Standard Deviation	CV (%)	Recovery (%)	*Bias (%)*
20^{o}C	10	0.003	0.0001	2.960	100.484	0.484
	10	0.063	0.0002	0.276	100.719	0.719
	10	0.125	0.0004	0.283	100.022	0.022
25^{o}C	10	0.003	0.0000	0.825	97.761	-2.239
	10	0.063	0.0004	0.651	101.338	1.338
	10	0.123	0.0007	0.603	98.686	-1.314
37^{o}C	10	0.003	0.0001	3.443	100.588	0.588
	10	0.063	0.0004	0.606	100.933	0.933
	10	0.125	0.0005	0.421	99.854	-0.146

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 -2.24 % to 1.34 %, 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 metabisulfite, and potassium disulfite. The expected concentration was 0.125 g/L.

Sulfite Form	Extraction Buffer	Reaction Time (mins)	Concentration (g/L)	Recovery (%)
Sodium Sulfite	0.1% Citric Acid	3	0.120	95.766
Sodium Bisulfite	0.1% Citric Acid	3	0.124	99.250
Sodium Metabisulfite	0.1% Citric Acid	3	0.119	95.208
Potassium Disulfite	0.1% Citric Acid	3	0.130	103.884

The assay demonstrated equivalent response to commonly used inorganic sulfite salts, with recoveries of 96-104%.

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 an internal standard. 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	103.35
D-Fructose	300	102.61
D-Galactose	5	101.83
D-Mannose	5	101.23
Sucrose	200	100.67
Lactose	5	100.87
Glycerol	20	101.23
Sorbitol	10	100.67
Malic Acid	50	100.05
Tartaric Acid	10	98.21
Citric Acid	50	100.14
Sucinnic Acid	5	100.12
Galacturonic Acid	10	101.56
Acetic Acid	10	100.02
Ascorbic Acid	10	122.16
	2.5	104.76
Sodium Chloride	10	100.26
Calcium Chloride	10	100.42
Ethanol	120	101.60
L-Cysteine	0.5	102.88
L-Gluthatione	0.2	102.66
Acetaldehyde	0.1	99.10
Taurine	1	101.45

* 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 ascorbic acid at concentrations higher than 2.5 g/L. All compounds tested met the recovery acceptance criteria of 100 ± 5% indicating that no significant cross-reactivity occurred with any of the other tested substances.

Matrix Interference

Matrix-related interference was assessed by spiking extracted samples from complex matrices with a validated aqueous sulfite standard at a concentration of 0.063 g/L and measuring the spike 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	0.065	0.064	97.987
White Wine	0.067	0.064	96.128
Rose Wine	0.064	0.063	98.592
Beer	0.064	0.064	99.360
Mean	0.065	0.064	98.017
Standard deviation	0.0011	0.000	1.379
CV (%)	1.742	0.672	1.406

A total of four 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.

* **Red Wine Samples:** During method evaluation, a progressive increase in signal response was observed when analyzing red wine samples. This signal drift can lead to overestimation of sulfite concentration if reaction times are not strictly controlled, as measured results may continue to increase during the assay. To minimize this effect, a fixed reaction time of 3 minutes ± 30 seconds must be used for all red wine analyses.

The extent of signal drift varies between individual red wine samples and is therefore matrix-dependent. Adjustment of red wine samples to 20% (v/v) ethanol prior to analysis was shown to significantly reduce this effect, resulting in acceptable spike recovery performance. This improvement is attributed to enhanced equilibrium between free and bound sulfur dioxide, leading to more stable free sulfite measurements.

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.

	Free Sulfite Recovery (g/L)			
Expected Free Sulfite (g/L)	T0	1st Cycle	2nd Cycle	3rd Cycle
0.003	0.003	0.003	0.003	0.003
0.063	0.065	0.061	0.061	0.063
0.125	0.124	0.121	0.121	0.123

The reagent demonstrated good stability across three repeated freeze–thaw cycles. Recoveries for all concentration levels remained within the 95–105 % acceptance criteria, and % CV values were all below 1%, indicating maintained precision following freeze–thaw exposure. Statistical analysis using single-factor ANOVA showed no significant differences between T0 and subsequent freeze–thaw cycles ($F = 0.0009 < F_{crit} = 4.0662$; $p = 0.9999$). These results confirm that repeated freeze–thaw cycling does not adversely impact reagent performance, supporting its robustness under the tested conditions.

Assay Temperature

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

	Free Sulfite Recovery (g/L)		
Expected Free Sulfite (g/L)	20°C	25°C	37°C
0.003	0.003	0.003	0.003
0.063	0.063	0.064	0.065
0.125	0.123	0.126	0.125
Reaction Time	3 min	3 min	3 min
Recommended time	*3-minute reaction time is recommended*		

Across the three sulfite levels tested (0.003, 0.063, and 0.125 g/L), recoveries at 20 °C and 37 °C were highly comparable to the 25 °C values, with only minor numerical differences 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 = 1.51) was well below the critical value (T_{crit} = 4.30), with a p-value of 0.30. Similarly, the 37 °C vs 25 °C comparison yielded a non-significant result (t = –97, p = 0.43). 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.

Free Sulfite (g/L)	Replicates, n	Mean (g/L)	Standard Deviation	% CV
0.003	24	0.003	0.0001	2.93
0.063	24	0.063	0.0007	1.16
0.125	24	0.123	0.0014	1.12

At the low (0.003 g/L), mid (0.063 g/L), and high (0.125 g/L) free 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 by 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	0.0140	0.0005	3.26
White Wine	12	0.0027	0.0002	8.57
Beer (Lager)	12	0.0006	0.0001	9.85
Rose Wine	12	0.0229	0.0007	3.12

Mean results for red wine, white wine, beer (lager), and rose wine fall within this range and show reasonable variability for most matrices. Precision was acceptable for red wine and rosé wine, while higher %CV values observed for white wine and beer at lower concentrations reflect increased variability under the precision limit. Overall, the results support the suitability of the method across the specified concentration range and matrices.

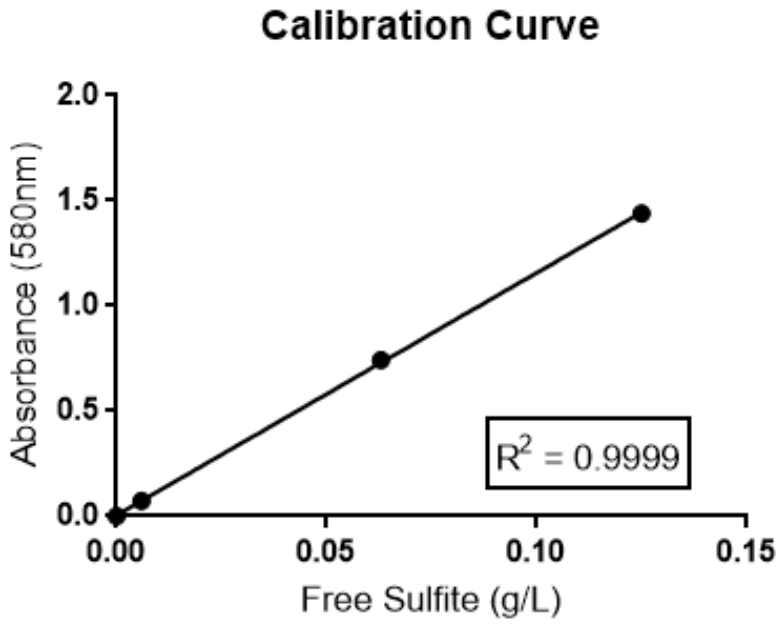
NOTE: Users should perform matrix validation work prior to routine use. This process will highlight any problematic matrices encountered. If you have questions about these or other matrices, please contact your local sales representative for support.

METHOD AUTOMATION

The Free 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 free sulfite is achieved through a single-test format using a cubic spline 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 free sulfite a calibration curve must be performed concurrently using the same batch of reagents.

Free Sulfite (g/L)	Replicates, n	Mean result (g/L)	Standard Deviation	% CV
0.006	3	0.0059	0.0007	7.91
0.063	3	0.0625	0.0028	4.44
0.125	3	0.1261	0.0040	3.14



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.