Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

Validation Report: Free Sulfite Assay Kit (cat. no. K-FSULPH)

1. Scope

Megazyme's Free Sulfite Assay Kit (K-FSULPH) is a reliable and accurate method used for the rapid measurement and analysis of total sulfite (sulfite) in wine, beverages, foodstuffs and other materials. This method measures Free sulfite in mg/L and is used widely in the wine industry. This method has been validated for red and white wines at the Bundesamt für Weinbau, Austria.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by Free Sulfite Assay Kit (K-FSULPH).

3. Performance characteristics

The selectivity, working range, limit of detection, limit of quantification, trueness (*bias*) and precision of this kit is detailed in this report.

3.1. Selectivity

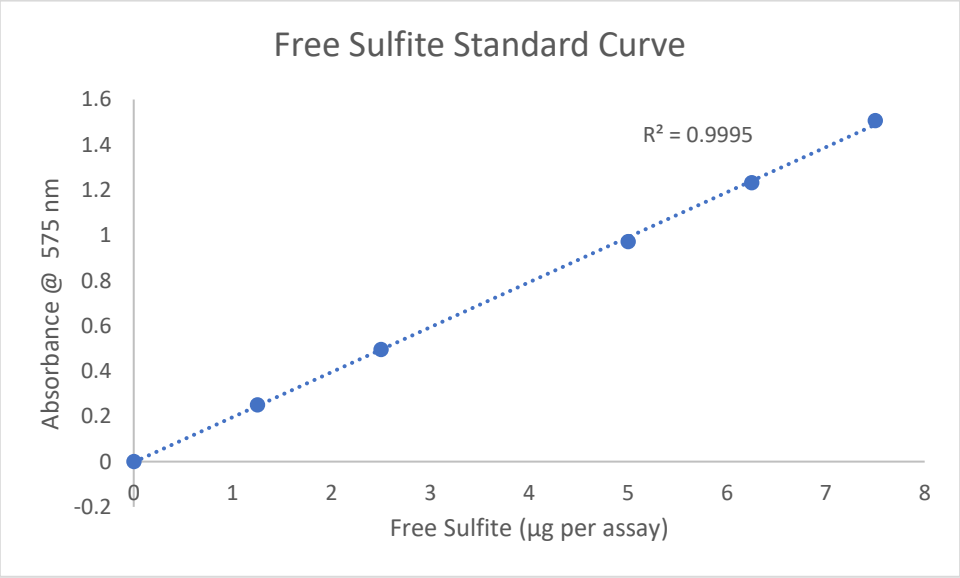
This assay is specific for free sulfite. Thiol reactive compounds (e.g. aldehydes, maleimide compounds) can interfere with the FSO_2 assay. Aldehyde levels in wine do not cause interference of the FSO_2 assay.

3.2. Working Range

Assay follows the Free Sulfite Assay Kit (K-FSULPH) standard procedure. 0.05 mL of sulfite standard was used as sample, with a range of concentrations (5-150 mg/L sulfite) which corresponds to 0.25-7.5 µg of sulfite per cuvette. Absorbance A2 was read after 3 min, and A3, after a subsequent 3 min, at 575 nm and at 25°C as recommended in the procedure.

Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

Free Sulfite Concentration [µg/assay]	ΔA_{575nm}
0	0.000
1.25	0.251
2.5	0.496
5.0	0.972
6.25	1.232
7.5	1.507



Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is ~ 2 mg/L of sulfite, which is derived from an absorbance difference of 0.020 with the maximum sample volume of 0.05 mL.

The **calculated limit of detection (LOD)** and the **calculated limit of quantification (LOQ)** for this report purpose is based on the analysis of samples that have been taken through the whole Free Sulfite Assay Kit (K-FSULPH) measurement procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as 3 x s'0; where s'0 is the standard deviation of a number of samples A1 reading.
- The LOQ is the lowest level at which the kit's performance is acceptably repeatable. LOQ is calculated as kQ x s'0; where s'0 is the standard deviation of a number of samples A1 reading. The IUPAC default value for kQ is 10.

- For Free Sulfite Assay Kit (K-FSULPH)

  LOD – For 0.05 mL of sample (maximum volume)
  Free Sulfite = 1.090 mg/L

  LOQ – For 0.05 mL of sample (maximum volume)
  Free Sulfite = 2.280 mg/L

* **Note:** The above detection limits are for samples as used in the assay, after sample preparations if required (e.g. deproteinisation). The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the Free Sulfite Assay Kit (K-FSULPH) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: ***b*(%) = x – xref / xref x 100**. The reference material for this purpose is sodium bisulphite supplied with the Free Sulfite Assay Kit (K-FSULPH) as FSO_2 at 150 mg/L.

Relative Bias *b*(%)

	n	Ref Material (mg/L)	Mean (mg/L)	*b*(%)
Free Sulfite	10	150	156.02	4.01

3.5. Precision

This report details the reproducibility of the Free Sulfite Assay Kit (K-FSULPH), it is a measure of the variability in results, on different days and by different analysts, over an extended period of time.

For the purpose of this report different lot numbers of the kit standard are used as the reference material.

Reproducibility

	n	Ref Material (mg/L)	Mean (mg/L)	Standard Deviation	%CV
Free Sulfite	10	150	156.02	7.4025	4.74

Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

Repeatability of this kit can be assessed using wine samples. This is a measure of the variability in results by a single analyst, using real samples, using the same equipment and over a short period of time. The use of wine samples shows one of the many applications of this kit.

Repeatability

	n	Mean (mg/L)	Standard Deviation	%CV
White Wine	12	22.43	1.07	4.76
Red Wine	12	43.16	1.29	2.98

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of free sulfite in various matrices. It has been used for many years and is fully automatable for high throughput analysis of samples. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	Free Sulfite
Working range (µg in cuvette)	0.25-7.5
LOD (mg/L)	1.090
LOQ (mg/L)	2.280
Relative Bias *b* (%)	4.01
Reproducibility (%CV using sodium bisulfite)	4.74
Repeatability (%CV using white wine)	4.76
Repeatability (%CV using red wine)	2.98