FREE SO_2

2 x 100 ml

INTENDED USE

Reagent for colorimetric determination of free sulphurous anhydride in foodstuff and other sample material.

PRINCIPLE OF THE METHOD

Free sulphurous anhydride reacts selectively with 4-[(4-aminophenyl)-(4-imino-2,5-cyclohexadiene-1-ylidene)methyl]-2-methybenzeneamine hydrochloride, producing a violet compound with a maximum of absorption at 578 nm. The amount of chromogenic compound formed is proportional to free SO_2 present in the sample.

KIT COMPONENTS

The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

SO2L R1: **2 x 80 ml (liquid) blue cap**
Composition: Buffer pH acidic, 4-[(4-aminophenyl)-(4-imino-2,5-cyclohexadiene-1-ylidene)methyl]-2-methybenzeneamine hydrochloride, stabilizers.

SO2L R2: **1 x 40 ml (liquid) red cap**
Composition: formaldehyde.

SO2L R3: **1 x 5 ml (liquid) white cap**
Composition: oxidizer.

Store all components at 2-8°C.

In vitro use only.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes.
Standard free SO_2 80 mg/l can be prepared in laboratory according to the following indication.
Materials required:
- deionized water,
- sodium metabisulfite (CAS 7681-57-4), pur. ≥ 99%,
- volumetric glassware class A

Procedure:
Place 1.23 g of sodium metabisulfite into a 100 ml volumetric flask, then add 40 ml of deionized water, and slowly swirl until complete dissolution. Fill up to the mark with deionized water.
Pour 1 ml of this solution into another volumetric flask, and bring to the volume of 100 ml with deionized water, then mix well. The standard is ready to use.
Store at 2-8°C.
Stability: prepare daily.

REAGENT PREPARATION

Procedure 1:
Use separate reagents.
Stability: until expiration date on the label at 2-8°C.

Procedure 2:
Working reagent: mix 4 parts of reagent R1 with 1 part of reagent R2.
Blank reagent: mix 8 parts of reagent R1 with 2 part of reagent R2 and add 0.25 parts of reagent R3.

It is suggested to prepare strictly the amount needed for the analysis, and any residue has to be stored at 2-8°C away from direct light sources.
Analytical performances of mixed reagent begin to fall off 48 hours after its preparation.

PRECAUTIONS

SO2L R1: **Danger.** Causes serious eye irritation (H319). Causes skin irritation (H315). Wear protective glover. Eye protection (P280). IF ON SKIN: Wash with plenty of water (P302+P352). IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing (P305+P351+P338).
Photosensitive reagent; keep away from direct light sources.

SO2L R2: **Danger.** May cause an allergic skin reaction (H317). May cause cancer (H350). Wear protective glover. Eye protection (P280). IF ON SKIN: Wash with plenty of water (P302+P352). If skin irritation or rash occurs: Get medical advice (P333+P313). Wash contaminated clothing before reuse (P363). IF exposed or concerned: Get medical advice (P308+P313). Do not handle until all safety precautions have been read and understood (P202).

SO2L R3: It is not classified as hazardous.

SPECIMEN

Wine or any foodstuff once its utilization has been tested.

PROCEDURE 1

Wavelength: 578 nm - accepted also 600 nm
Lightpath: 1 cm
Temperature: 37°C

dispense:	reagent blank	standard	sample blank	sample
standard	-	50 µl	-	-
sample	-	-	50 µl	50 µl
reagent R3	-	-	50 µl	-
reagent R1	1600 µl	1600 µl	1600 µl	1600 µl
water	100 µl	50 µl	-	50 µl
reagent R2	400 µl	400 µl	400 µl	400 µl

Mix, incubate at 37°C for 10 minutes.
Read absorbances of standard (As), sample (Ac), sample blank (Abc) and reagent blank (Ar).

RESULTS CALCULATION

$$\text{Free } SO_2 \text{ mg/l} = \frac{Ac - Abc}{As - Ar} \times \text{standard value}$$

If standard was not available, make the calculation as follows:

Free SO_2 mg/l = (Ac - Abc) x 70.5

PROCEDURE 2 (cell flow instruments)

Wavelength: 578 nm - accepted also 600 nm
Lightpath: 1 cm
Temperature: 37°C

dispense:	reagent blank	standard	sample blank	sample
water	50 µl	-	-	-
standard	-	50 µl	-	-
sample	-	-	50 µl	50 µl
reagent	2 ml	2 ml	-	2 ml
blank	-	-	2 ml	-

Mix, incubate at 37°C for 10 minutes.
Read absorbances of standard (As), sample (Ac), sample blank (Abc) and reagent blank (Ar).

RESULTS CALCULATION

$$\text{Free } SO_2 \text{ mg/l} = \frac{Ac - Abc}{As - Ar} \times \text{standard value}$$

If standard was not availale, make the calculation as follows:

Free SO_2 mg/l = (Ac - Abc) x 69.0

TEST PERFORMANCE

Specificity
The method is specific for free sulphurous anhydride.

Linearity
The method is linear up to 150 mg/l.
If the limit value is exceeded, it is suggested to dilute the sample 1+4 with distilled water and to repeat the test, multiplying the result by 5.

Precision
White wine
intra-assay (n=10)

	mean (mg/l)	SD (mg/l)	CV%
sample	2.90	0.10	3.33

inter-assay (n=20)

	mean (mg/l)	SD (mg/l)	CV%
sample	2.88	0.22	7.83

Rose wine
intra-assay (n=10)

	mean (mg/l)	SD (mg/l)	CV%
sample	2.88	0.07	2.37

inter-assay (n=20)

	mean (mg/l)	SD (mg/l)	CV%
sample	2.83	0.23	7.95

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

W.M. Grant, Anal. Chem., 19, 345 (1947).
A. Steigmann, Anal. Chem., 22, 492 (1950).
M.A. Joslyn, Am. J. Enol. Vitic., 6, 1-10 (1955)

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SYMBOLS

LOT lot of manufacturing
REF code number
storage at temperature interval
expiration date (year/month)
warning, read enclosed documents
read the directions

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