TOTAL SULPHITE (TSO2)

Colorimetric – Method
RX ALTONA
MANUAL
FOOD AND WINE

INTENDED USE

For the quantitative determination of Total Sulphite in wine. This product is suitable for manual use and on the RX **altona** analyser. **Applications for a variety of additional analysers are available from www.randoxfooddiagnostics.com.**

FOR THE ANALYSIS OF FOOD AND WINE. Not for diagnostic procedures.

Cat. No.			
TS4051	R1.	Buffer	1 x 100 ml
	R2.	Chromogen	1 x 3 ml
		CAL BLANK	1 x 1 ml
		CALa	2 x 32 mg
		CALb	2 x 40 ml

SIGNIFICANCE

All wines contain sulphur dioxide (SO_2) in various forms, collectively known as sulphites. During the wine making process, SO_2 is used as an essential additive, predominantly for its suppression of yeast and bacterial action and its anti-oxidant properties. SO_2 is present in wine in unbound (free) and bound forms. Only free SO_2 is active as an antimicrobial and antioxidant preservative. Given that a proportion of SO_2 added to wine becomes inactive when it binds to components such as polyphenolics and sugar, and with legal restrictions on SO_2 levels in wine, it is useful for wine producers to quantify both Free SO_2 and Total SO_2. This kit is suitable for the quantification of Total (free and bound) SO_2 in wine.

PRINCIPLE

This end-point colorimetric test is based on the principle that at neutral pH bound sulphites dissociate and react with Ellman's reagent to produce a coloured reaction product which is measured photometrically at 415 nm. The absorbance from polyphenols and wine pigments is corrected for by sample blanking.

SAMPLE

Red wine, white wine and fruit juices. Turbid samples should be filtered prior to assay. Strongly coloured samples should be decolourised with 0.2 g polyvinylpolypyrrolidone (PVPP) to approximately 10 ml of juice or wine. Shake vigorously for 5 minutes and filter. The clear filtrate can then be used in the assay undiluted.

SAFETY PRECAUTIONS AND WARNINGS

For the analysis of food and wine. Not for diagnostic procedures. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solutions R1and R2 contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety data sheets are available on request.

Please dispose of all Biological and Chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1. Buffer
Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

R2. Chromogen
Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

CAL BLANK Reagent Blank (S0)
Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

CALa
Stable up to the expiry date specified when stored at +2 to +8°C.

CALb
Stable up to the expiry date specified when stored at +2 to +8°C.

CAL Calibrator (S1)
Tap the lid of CALa several times to ensure that all powder is transferred from the lid and bung to the glass bottom of the vial. Carefully remove the lid and bung from CALa, ensuring that no powder is lost in the process. Transfer 1ml of CALb to CALa using a micropipette and use the pipette tip to carefully mix the contents of the vial. Transfer the solution from CALa back into the CALb bottle, using the same pipette tip. Repeat this process two additional times to ensure that all powder has been completely dissolved and all solution transferred back into the CALb bottle. Seal the CALb lid tightly and gently swirl by hand for approximately 20 seconds to mix. Stable for 24 hours at +15 to +25°C when stored tightly sealed.

Sulphite in solution is not stable and will decrease in concentration over time. For greatest accuracy, the calibrator should be prepared immediately prior to use on day of analysis.

MATERIALS PROVIDED

Buffer
Chromogen
CAL BLANK
CALa
CALb

MATERIALS REQUIRED BUT NOT PROVIDED

Sulphite Calibrator Set (Cat no. TS4052)

RX ALTONA PROCEDURE

Select TSO2 in the Test Screen. Then select Run Calibration or Run Sample and carry out a water blank as instructed.

Pipette into cuvette:

	Reagent Blank	Standard	Sample
Sample	---	---	20 µl
CAL BLANK	20 µl	---	---
Standard (S1)	---	20 µl	---
Buffer (R1)	800 µl	800 µl	800 µl

Mix, and incubate for 3 minutes at +25°C. Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample Blank and press Read. Then add

Chromogen (R2)	20 µl	20 µl	20 µl

Mix, and incubate for 3 minutes at +25°C. Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample and press Read.

CALIBRATION FOR RX MONZA

A 2 point linear calibration is recommended with change in reagent lot or as indicated by quality control procedures. Use CAL BLANK and CAL supplied with kit.

CALIBRATOR CONCENTRATION

CAL BLANK	S0	0 mg/l
CAL	S1	406.6 mg/l

FOR MANUAL USE
PROCEDURE SEMI MICRO

Wavelength:	415 nm (405-420 nm)
Cuvette:	1 cm path length
Temperature:	+20 to +25°C
Measurements:	Against Reagent Blank

Pipette into 1 ml cuvette

	Blank	Standard	Sample
Buffer (R1)	1000 µl	1000 µl	1000 µl
CAL BLANK	25 µl	-	-
Standard (S1)	-	25 µl	-
Standard	-	-	25 µl

Mix, and read absorbance A_1 after 3 minutes.

Chromogen (R2)	25 µl	25 µl	25 µl

Mix and read absorbance A_2 after 3 minutes.

PROCEDURE MACRO

Wavelength:	415 nm (405-420 nm)
Cuvette:	1 cm path length
Temperature:	+20 to+25°C
Measurements:	Against Reagent Blank

Pipette into cuvette

	Blank	Standard	Sample
Buffer (R1)	2000 µl	2000 µl	2000 µl
CAL BLANK	50 µl	-	-
Standard (S1)	-	50 µl	-
Standard	-	-	50 µl

Mix, and read absorbance A_1 after 3 minutes.

Starter 3	50 µl	50 µl	50 µl

Mix and read absorbance A_2 after 3 minutes.

MANUAL CALCULATION

Determine absorbance differences $A_2 - A_1$, for blank and sample.

$$\Delta A = \Delta A_{\text{sample}} - \Delta A_{\text{blank}}$$

$$\text{Concentration} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{conc. of standard}$$

SPECIFIC PERFORMANCE CHARACTERISTICS

The following Total Sulphite performance characteristics were obtained using an RX **altona** analyser in cuvette mode at +25°C.

LINEARITY

The total sulphite assay is linear to 500 mg/l.

SENSITIVITY

The minimal detectable concentration of total sulphite with an acceptable level of precision was determined as 5.2 mg/l.

PRECISION

Intra assay precision

	Level 1	Level 2	Level 3
Mean (mg/l)	134.3	236.5	358.7
S.D	6.252	4.357	13.282
C.V. (%)	4.66	1.84	3.70
n	20	20	20

Inter assay precision

	Level 1	Level 2	Level 3
Mean (mg/l)	133.6	235.9	354.4
S.D	8.620	6.943	15.396
C.V. (%)	6.45	2.94	4.34
n	20	20	20

27 Sep 16 ml
Rev. 002

Randox Laboratories Ltd, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom
T +44 (0) 28 9442 2413 **F** +44 (0) 28 9445 2912 **E** applications@randox.com **I** www.randox.com