COD 12806 200 mL
Only for *in vitro* use in the laboratory

BioSystems REAGENTS & INSTRUMENTS

TOTAL SULFITE

5-5'-DITIO-2-NITROBENZOIC ACID (DTNB)

INTENDED USE

Reagent for the measurement of total sulfite in several types of samples.

PRINCIPLE OF THE METHOD

Total sulfites in the sample react with 5-5'-ditio-2-nitrobenzoic acid (DTNB) in a basic medium. The cleavage of disulfide bonds (R-S-S-R) of DTNB by a sulfite molecule generates 5-mercapto-2-nitrobenzoate that absorbes at 405 nm. The increase of the coloration is proportional to total sulfites in the sample[1,2].

$$SO_2 + \text{R-S-S-R (DTNB)} \xrightarrow{\text{pH = 8.2}} \text{R-S-SO}_2 + \text{S-R}$$

CONTENTS AND COMPOSITION

A. Reagent. 2 x 100 mL. Decolorant.

DANGER: H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

B. Reagent. 4 x 40 mL. Buffer solution. pH = 8.2.

C. Reagent. 1 x 40 mL. 5-5'-ditio-2-nitrobenzoic acid (DTNB). pH = 8.2.

S. Standard. 1 x 5 mL. Sulfite 150 mg/L. Aqueous primary standard.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE AND STABILITY

Store at 2-8 ºC.

Components are stable once opened until the expiry date marked in the label if they are stored well closed and care is taken to prevent contamination during their use.

Indications of deterioration: Absorbance of the blank over the limit indicated in "Test Parameters".

ADDITIONAL EQUIPMENT

Analyzer, spectrophotometer or photometer able to read at 405 nm.

REAGENT PREPARATION

Reagents and Standard (ST1) are provided ready to use.

Standard 15 mg/L (ST2): Dilute 1000 µL of Standard S in a 10 mL volumetric flask with distilled water. Stable for 30 days at 2-8ºC.

PROCEDURE (Notes 1 and 2)

Sample preparation

1. Pipette into a tube:

Sample (ST1)	200 µL
Reagent A	200 µL

2. Mix gently. Diluted sample is stable for 30 minuts at 15-25ºC.

Manual procedure

1. Pipette into a cuvette:

	Reagent Blank (RB)	Standard / Sample
Standard / Sample (ST1/ST2)	-	24 µL/134 µL
Distilled water (ST1/ST2)	24 µL/134 µL	-
Reagent B	800 µL	800 µL

2. Mix and incubate for 2 minutes at room temperature. Read absorbance (A1) at 405 nm.
3. Pipette into the cuvette:

Reagent C	200 µL	200 µL

4. Mix and incubate for 5 minutes at room temperature. Read the absorbance (A2) of the reagent blank, the standard and the sample at 405 nm. The color is stable during 15 minutes.
5. Calculate the total sulfite concentration using the following formula:

$$\frac{(A2 - 0.80 \times A1)_{Sample} - (A2 - 0.80 \times A1)_{RB}}{(A2 - 0.80 \times A1)_{Standard} - (A2 - 0.80 \times A1)_{RB}} \times C_{Standard}\,[mg/L] \times 2 = C_{Sample}\,(ST1)\,[mg/L]$$

$$\frac{(A2 - 0.83 \times A1)_{Sample} - (A2 - 0.83 \times A1)_{RB}}{(A2 - 0.83 \times A1)_{Standard} - (A2 - 0.83 \times A1)_{RB}} \times C_{Standard}\,[mg/L] = C_{Sample}\,(ST2)\,[mg/L]$$

CALIBRATION

A reagent blank should be done every day and a calibration after reagent lot change or as required by quality control procedures.

QUALITY CONTROL

Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS[3-5]

The metrological characteristics described below have been obtained using a Y15 analyzer. Details on evaluation data are available on request.

- Detection limit: 3 mg/L (ST1); 0.2 mg/L (ST2).
- Linearity limit: 400 mg/L (ST1); 38 mg/L (ST2).
- Precision:

Mean concentration (ST1)	Repeatability (CV)	Within-laboratory (CV)
49 mg/L	1.9 %	2.3 %
96 mg/L	0.5 %	1.3 %

Mean concentration (ST2)	Repeatability (CV)	Within-laboratory (CV)
5.1 mg/L	0.9 %	1.4 %
10.9 mg/L	0.4 %	1.0 %

– Trueness: Results obtained with this procedure did not show systematic differences when compared with a reference procedure. Details of the comparison experiments are available on request.

NOTES

1. Volumes proposed are to use a semi-micro cuvette. Other volumes can be used if the ratio between the reagents and sample is maintained.
2. The procedure and test parameters may vary depending on the sample type (Sample Type: ST).

BIBLIOGRAPHY

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4. Clinical and Laboratory Standards Institute (CLSI). Evaluation of detection capability for clinical laboratory measurement procedures; Approved guideline – Second edition. EP17-A2. Wayne: USA 2012.
5. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the linearity of quantitative measurement procedures: a statistical approach; Approved guideline – Third edition. EP6-A. Wayne: USA 2003.
6. International organization of vine and wine (OIV), Compendium of international methods of wine and must analysis Vol. 1 & 2, 2016.
7. Zoecklein BW, Fugelsang KC, Gump BH, Nury FS, Wine analysis and production. Van Nostrand Reinhold; 1 edition (December 31, 1990).
8. EBC Analysis Comitee. Analytica-EBC. Verlag Hans Carl; 7th edition (2010).

SAMPLES

Preparation procedures

- Sulfite is volatile and can be easily oxidized. Treat the samples accordingly.
- Filter or centrifuge turbid solutions.
- Dilute accordingly with distilled water samples with concentration over the specified linearity limit. Multiply obtained concentration by the dilution factor.

Red and white wine and must[6,7] (ST1): Filter and/or dilute wine if necessary as described in "Preparation procedures".

It is recommended to use the Sulfite Control I, II (BioSystems ref. 12827) to verify the performance of the measurement procedure.

Beer[8] (ST2): Degas, filter, clarify and/or dilute beer if necessary as described in "Preparation procedures".

Further applications: The method may also be used with other types of samples. Contact your supplier for more information.

TEST PARAMETERS (Note 2)

These reagents may be used in several automatic analysers. Specific instructions for application in many of them are available on request.

BioSystems Y15

Reagent 1 (Vol. R1): Use Reagent B.
Reagent 2 (Vol. R2): Use Reagent C.

GENERAL	
Test name	**TOTAL SULFITE**
Analysis mode	differential bireagent
Sample type	ST1 / ST2
Units	mg/L
Reaction type	increasing
Decimals	0 / 1
PROCEDURE	
Reading	monochromatic
Sample	7 / 40
Reagent 1	240
Reagent 2	60
Washing	1.2
Predilution factor	2 / -
Main	405
Reference	-
Reading 1	72 s
Reading 2	312 s
Reagent 2	96 s
CALIBRATION	
Calibration type	specific
Calibration curve	-
OPTIONS	
Blank Absorbance limit	0.500
Kinetic blank limit	-
Linearity limit	400 / 38

BioSystems Y25

Reagent 1 (Vol. R1): Use Reagent B.
Reagent 2 (Vol. R2): Use Reagent C.

GENERAL	
Test name	**TOTAL SULFITE**
Analysis mode	differential bireagent
Sample type	ST1 / ST2
Units	mg/L
Reaction type	increasing
Decimals	0 / 1
PROCEDURE	
Reading	monochromatic
Sample	7 / 40
Reagent 1	240
Reagent 2	60
Washing	1.2
Predilution factor	2 / -
Main	405
Reference	-
Reading 1	75 s
Reading 2	300 s
Reagent 2	90 s
CALIBRATION	
Calibration type	specific
Calibration curve	-
OPTIONS	
Blank Absorbance limit	0.500
Kinetic blank limit	-
Linearity limit	400 / 38

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www.biosystems.es *Quality System certified according to EN ISO 13485 and EN ISO 9001 standards*