

RANDOX TOTAL ANTIOXIDANT STATUS (TAS) MANUAL

INTENDED USE

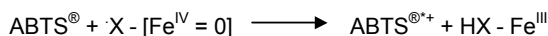
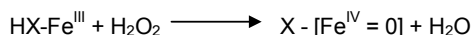
For the quantitative *in vitro* determination of Total Antioxidant Status in serum, plasma, wine, beer and fruit juice. This product is suitable for Manual use.

Cat No.

NX 2332	R1. Buffer	1 x 100 ml
5 x 10 ml	R2. Chromogen	5 x 10 ml
5 x 10 t	R3. Substrate	2 x 5 ml
	CAL. Standard	5 x 1 ml

ASSAY PRINCIPLE

ABTS[®] (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H₂O₂ to produce the radical cation ABTS^{®•+}. This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration.



HX-Fe^{III} = Metmyoglobin

X - [Fe^{IV} = O] = Ferrylmyoglobin

ABTS[®] = 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]

ABTS[®] is a registered trademark of Boehringer Mannheim.

SAMPLE ⁽²⁾

Freshly drawn serum or heparinized plasma. Avoid haemolysed samples. Sample may be stored for up to 36 hours at +2 to +8°C. Plasma/serum may be frozen for up to 14 days. Avoid repeated freeze thaw cycles. Red wine - dilute 1 + 3. Fruit juices can also be assayed, but must be filtered using a 0.45 µl filter.

REAGENT COMPOSITION

Contents	Concentrations in the Test
R1. Buffer	
Phosphate Buffered Saline	80 mmol/l, pH 7.4
R2. Chromogen	
Metmyoglobin	6.1 µmol/l
ABTS [®]	610 µmol/l
R3. Substrate	
Hydrogen peroxide (in stabilised form)	250 µmol/l
CAL. Standard	
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid	lot specific

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

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 expiry date when stored at +2 to +8°C.

R2. Chromogen

Reconstitute one vial of chromogen R2 with **10 ml** of Buffer R1. Stable for 2 days at +2 to +8°C or 8 hours at +15 to +25°C.

R3. Substrate

Dilute 1 ml of substrate R3 with **1.5 ml** Buffer R1. Stable for 24 hours when stored at +2 to +8°C. Stable undiluted up to expiry date when stored at +2 to +8°C.

CAL. Standard

Reconstitute one vial of Standard with **1 ml** of double deionized water. Stable for 2 days at +2 to +8°C or 1 month at -20°C.

N.B.: If using this assay on an automated system, please refer to procedure sheet for that system as reconstitution instructions may be different.

MATERIALS PROVIDED

Buffer
Chromogen
Substrate
Standard

MATERIALS REQUIRED BUT NOT PROVIDED

Radox Total Antioxidant Control (Cat. No. NX 2331).

NOTE

It is important to time the reaction as accurately as possible. If volumes, incubation times and temperatures are changed this will affect the results of the assay. Total Antioxidant Status is only suitable for use on a temperature-controlled spectrophotometer.

PROCEDURE

Wavelength:	600 nm
Cuvette:	1cm light path
Temperature:	37°C
Measurement:	against air

Pipette into cuvette:

	Reagent Blank	Standard	Sample
DDH ₂ O	20 µl	-	-
Standard	-	20 µl	-
Sample	-	-	20 µl
Chromogen (R2)	1 ml	1 ml	1 ml

Mix well, incubate to bring to temperature and read initial absorbance (A₁)

Add:

Substrate (R3)	200 µl	200 µl	200 µl
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Mix and start timer simultaneously.
Read absorbance after exactly 3 minutes (A₂)

A₂ - A₁ = ΔA of sample/standard/blank

CALCULATION

Total Antioxidant Status:

$$\text{Factor} = \frac{\text{conc of standard}}{(\Delta A \text{ blank} - \Delta A \text{ standard})}$$

$$\text{mmol/l} = \text{Factor} \times (\Delta A \text{ Blank} - \Delta A \text{ Sample})$$

QUALITY CONTROL

Randox Total Antioxidant Control is recommended for daily quality control. The control should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Support, Northern Ireland (028) 94422413.

REFERENCE RANGES

Range: 1.30 - 1.77 mmol/l Plasma

This range was measured in an European working population. It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

LINEARITY

Samples with concentrations greater than 2.5 mmol/l should be diluted with 0.9% NaCl and reassayed. Dilution of sample results in up to a 20% increase in values and so is only recommended if absolutely necessary. The majority of samples will not require dilution as the results will be less than 2.5 mmol/l.

PATENTS

This product is the subject of UK Patent 2250819 and Patents and Applications deriving from PCT Patent Application PCT/GB91/02228.

REFERENCES

1. Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V. and Milner, A., Clinical Science (1993) **84**, 407-412.
2. Data on file at Randox.

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