TOTAL ANTIOXIDANT STATUS (TAS)

MANUAL

INTENDED USE

For the quantitative *in vitro* determination of Total Antioxidant Status in serum, plasma, beer and fruit juice. This product is suitable for Manual use. **Applications for a variety of additional analysers are available from www.randoxfooddiagnostics.com.**

Cat No.			
NX 2332	R1.	Buffer	1 x 100 mL
5 x 10 mL	R2.	Chromogen	5 x 10 mL
5 x 10 tests	R3.	Substrate	2 x 5 mL
	CAL.	Standard	5 x 1 mL

GTIN: 05055273204735

ASSAY PRINCIPLE[1]

ABTS® (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H_2O_2 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.

$$HX\text{-}Fe^{III} + H_2O_2 \longrightarrow X\text{ - }[Fe^{IV} = 0] + H_2O$$

$$ABTS® + {}^{\cdot}X\text{ - }[Fe^{IV} = 0] \longrightarrow ABTS®^{*+} + HX\text{ - }Fe^{III}$$

HX-Fe^{III} = Metmyoglobin
X - [Fe^{IV}= 0] = Ferrylmyoglobin
ABTS® = 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]
ABTS® is a registered trademark of Boehringer Mannheim.

SAMPLE [2]

Freshly drawn serum or heparinised 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.
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 deionised 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

Randox Total Antioxidant Control (Cat. No. NX 2331)
0.9% NaCl Solution (Without Sodium Azide)

NOTES

1. It is important to time the reaction as accurately as possible. If volumes and incubation times are changed this will affect the results of the assay. Total Antioxidant Status is only suitable for use on a temperature-controlled spectrophotometer.
2. Sodium Azide interferes in the assay and should not be added to the 0.9% NaCl solution, which may be used to dilute samples which exceed the linearity of the assay.

PROCEDURE

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

Pipette into cuvette:

	Reagent Blank	Standard	Sample
DDH_2O	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 (A_1)
Add:

Substrate (R3)	200 µL	200 µL	200 µL

Mix and start timer simultaneously.
Read absorbance after exactly 3 minutes (A_2).

$A_2 - A_1 = \Delta A$ of sample/standard/blank

CALCULATION

Total Antioxidant Status:

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

mmol/l = Factor x (ΔA Blank - ΔA 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 Services, Northern Ireland +44 (0) 28 9445 1070.

INTERFERENCES

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances[3]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

REFERENCE RANGES[2]

Serum: 1.30 - 1.77 mmol/L

This range was measured in a 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 re-assayed. 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.
3. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.

The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

Revised 11 Jul 22 ld