RANDOX

TOTAL ANTIOXIDANT STATUS (TAS)

MANUAL
RX MONZA

FOR FULL PRODUCT DETAILS, PLEASE REFER TO THE KIT INSERT

INTENDED USE

For the quantitative analysis of Total Antioxidant Status in wine and fruit juice. This product is suitable for Manual use and on the RX **monza**.

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

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

SAMPLE

White, Rose and Red wine samples should be diluted appropriately according to the dilution table provided. For greatest accuracy, pre-dilutions should result in a concentration between 0.5-2 mmol/L. Fruit juices can also be assayed, but must be filtered using a 0.45 µL filter. Samples containing significant amount of carbon dioxide should be degassed prior to analysis by stirring with glass rod.

NOTE

Analysis of 20 wine samples reviled 6 out of 7 white wine samples could be analysed neat. 5 out of 6 rose wine samples had to pre-dilute 1:3 prior to analysis. 4 out of 7 red wines had to be pre-diluted 1:12 with the other 2 red wine samples being further pre-diluted 1:20 prior to analysis.

It is recommended that laboratory's should establish their own reference range for Total Anti-Oxidant levels in wines for their region.

Dilution Table

Estimated concentration of TAS (mmol/L)	Dilution with water	Dilution Factor(F)
0.2-2	No dilution required	1
0.6-6	1:3	3
1.2-12	1:6	6
2.4-24	1:12	12
4-40	1:20	20

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

DDH_2O

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

PROCEDURE

Select an open channel on the Run Test screen, enter the assay parameters exactly as they appear on next page and save. Select Run and carry out water blank as instructed.

Pipette into cuvette:

	Reagent Blank	Standard	Sample
DDH_2O	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL
Chromogen (R2)	500 µL	500 µL	500 µL

Mix, and incubate for 2 minutes at +37°C. Insert the cuvette into the RX **monza** flowcell holder when prompted and press Read. Then add:

Substrate (R3)	100 µL	100 µL	100 µL

Mix and start timer simultaneously. Place into RX **monza** flowcell holder after exactly 3 minutes press Read.

RX MONZA CALIBRATION

A 2 point linear using 1 replicate calibration is recommended with change in reagent lot or as indicated by quality control procedures. Use CAL Standard provided in the kit. Refer to attached sheet for details to be entered for calibration on an RX **monza** analyser.

FOR MANUAL USE

Wavelength:	600 nm
Cuvette:	1cm 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)

CALIBRATION

A 2 point linear using 1 replicate calibration is recommended with change in reagent lot or as indicated by quality control procedures. Use CAL Standard provided in the kit. Refer to attached sheet for details to be entered for calibration on an RX **monza** analyser.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following Total Anti-Oxidant performance characteristics were obtained using a RX **monza** analyser in cuvette mode at +37°C.

LINEARITY

The method is linear to a Trolox concentration of 2.58 mmol/L. Wine samples with concentrations greater than 2 mmol/L should be pre-diluted with deionised water accordingly and re-assayed.

SENSITIVITY

The minimal detectable concentration of Trolox with an acceptable level of precision was determined as 0.2 mmol/L.

PRECISION

Within Run precision

	White Wine	Red Wine
Mean (mmol/L)	1.57	22.99
S.D	0.062	0.050
C.V(%)	3.98	2.62
N	20	20

Total Run precision

	White Wine	Red Wine
Mean (mmol/L)	1.64	23.69
S.D	0.099	0.074
C.V(%)	6.06	3.77
n	20	20

Monza Parameters

Report Name	Delay Time	Cuvette
TAS	2 sec	10 mm CUVETTE

Assay Mode	Read Time	Ref Low
1-PT-S-BLANK	1 sec	

Pri Wavelen	Unit	Ref High
600 nm	mM/L	

Sec Wavelen	Format	Min Lin Lim
NONE	#.###	0.200

Temperature	Replicates	Max Lin Lim
37°C	1	2.500

%Linearity	Asp Volume	Slope a
	-	1.0000

Min RX Abs	Samp volume	Intercept b
	10 µL	0.0000

Max RX Abs	R1 volume	Assay Name2
	500 µL	

Min Rgt Abs	R2 Volume	Report Name2
	100 µL	

Max Rgt Abs	R3 Volume

C1 Mean	C2 Mean	C3 Mean

C1 2SD	C2 2SD	C3 2SD

Monza Calibration

Date & Time	Curve Type	Repl
	LINEAR	1

Standard	Conc.	ΔA/min	Factor
S0	0		
S1	*		
S2			
S3			
S4			
S5			
S6			
S7			
S8			
S9			
S10			
S11			

± Repl Lim	± Fact Dev%	Curve Fit Lim

Curve Fit-R

*Data entered by operator

23 Apr 13 rw