

CITRIC ACID

Colorimetric – Method

RX ALTONA

MANUAL

FOOD AND WINE

INTENDED USE

For the quantitative determination of Citric Acid in food and 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.

CTR4065	R1.	Enzyme Reagent	4 x 10 ml
	R2.	Citrate Lyase	4 x 1 ml
	R3.	Buffer	1 x 41 ml
	CAL	Standard	1 x 5 ml

SIGNIFICANCE

Citric acid is a weak, organic acid which occurs naturally in large quantities in citric fruits. In grapes however it is relatively minor acid, making up approximately 5% of the total acid content. Citric acid can be added to wine by winemakers to boost wine acidity or act as a stabilising agent to help prevent ferric hazes. Within the EU citric acid can only be used for stabilisation purposes and final citric acid content should not exceed 1g/L.

PRINCIPLE

Citric acid is converted to oxaloacetate and acetate in a reaction catalysed by citrate lyase. In the presence of malate dehydrogenase and lactate dehydrogenase, oxaloacetate and its decarboxylation derivative pyruvate are reduced to L-malate and L-lactate by NADH. The amount of NADH oxidised to NAD⁺ is proportional to the amount of citrate present. The oxidation of NADH is measured by the resultant decrease in absorbance at 340nm.

SAMPLE

Wine. Turbid samples should be filtered prior to assay. Strongly coloured samples should be treated with 0.6g PVPP to 10mL of wine, shaken vigorously and filtered prior to analysis. Sparkling wine samples should be degassed before analysis.

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 R3 and CAL 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. Enzyme Reagent

Reconstitute 1 vial with 10mL R3 buffer. Stable for 5 days at +2 to +8°C or 12 weeks at -20°C when stored in glass vial.

R2. Citrate Lyase

Reconstitute 1 vial with 1mL ddH₂O. Stable for 3 days at +2 to +8°C or 12 weeks at -20°C when stored in glass vial.

R3. Buffer

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

CAL Standard

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

MATERIALS PROVIDED

Enzyme Reagent
Citrate Lyase
Buffer
CAL

MATERIALS REQUIRED BUT NOT PROVIDED

Double deionised / distilled water

RX ALTONA PROCEDURE

Select Citric Acid in the Test screen. Then select Run Calibration or Run Sample and carry out a water blank as instructed.

Pipette into cuvette:

	S0	S1	Sample
ddH ₂ O (S0)	50 µl	---	---
CAL (S1)	---	50 µl	---
Sample	---	---	50 µl
Enzyme Reagent (R1)	500 µl	500 µl	500 µl

Mix, and incubate for 5 minutes at +25°C. Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample Blank and press Read. Then add:

Citrate Lyase (R2)	50 µl	50 µl	50 µl
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Mix, incubate for a further 5 minutes at +25°C. Insert the cuvette into the RX **altona** flowcell holder when prompted for Sample and press Read.

CALIBRATION FOR RX ALTONA

A 2 point linear calibration is recommended with change in reagent lot or as indicated by quality control procedures. Use CAL Standard provided in the kit.

FOR MANUAL USE PROCEDURE SEMI MICRO

Wavelength:	340 nm
Cuvette:	1 cm path length
Temperature:	+20 to +25°C
Measurements:	Against Reagent Blank

Pipette into 1 ml cuvette

	S0	S1	Sample
ddH ₂ O (S0)	100 µl	---	---
CAL (S1)	---	100 µl	---
Sample	---	---	100 µl
Enzyme Reagent (R1)	1000 µl	1000 µl	1000 µl

Mix, and read absorbance A1 after 5 minutes at +25°C. Then add

Citrate Lyase (R2)	100 µl	100 µl	100 µl
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Mix, and read absorbance A2 after 5 minutes at +25°C.

MANUAL CALCULATION

Calculate A2 - A1 for all standards/samples (ΔA).
Plot a linear calibration graph using ΔA standards (x axis) versus Standard Conc. mg/l (y axis).
Use the linear regression equation achieved ($y = mx + c$) to calculate the citric acid concentration for samples, where y = concentration (mg/l) and x = ΔA sample.

SPECIFIC PERFORMANCE CHARACTERISTICS

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

LINEARITY

The citric acid assay is linear to 400 mg/l.

SENSITIVITY

The minimal detectable concentration of citric acid with an acceptable level of precision was determined as 9.6 mg/l.

PRECISION

Intra assay precision

	Level 1	Level 2	Level 3
Mean (mg/l)	82.0	221.3	250.5
S.D	2.087	3.926	4.577
C.V(%)	2.55	1.77	1.83
n	20	20	20

Inter assay precision

	Level 1	Level 2	Level 3
Mean (mg/l)	79.6	218.5	260.5
S.D	3.522	5.100	8.118
C.V(%)	4.43	2.33	3.12
n	20	20	20