EN

CITRIC ACID

REF 984327

❶	3 x 20 ml Buffer A (Ready to use)
❷	3 x vial Lyo A (Lyophilisate)
❸	1 x 20 ml Buffer B (Ready to use)
❹	4 x vial Lyo B (Lyophilisate)
❺	Empty 10 ml vial

INTENDED USE
Reagent for photometric determination of free Citric acid in homogenous liquid samples using automated Thermo Scientific™ Arena™ or Gallery™ analyzer.

Reagent kit can also be used for manual pipetting procedure.

METHOD
Enzymatic test with citrate lyase.
Method is performed at 37 °C, using 340 nm filter.

PRINCIPLE OF THE PROCEDURE
In the first reaction Citric acid (citrate) is converted to oxaloacetate and acetate in the reaction catalyzed by the enzyme citrate lyase (CL).

Citrate -----CL----> oxaloacetate + acetate

In the presence of the enzymes L-malate dehydrogenase (L-MDH) and L-lactate dehydrogenase (L-LDH), oxaloacetate and its decarboxylation product pyruvate are reduced to L-malate and L-lactate, respectively, by reduced nicotinamide-adenine dinucleotide (NADH).

Oxaloacetate + NADH + H+ ---- L-MDH ---> L-malate + NAD+
Pyruvate + NADH + H+ ----- L-LDH ----> L-lactate + NAD+

The amount of NADH oxidized in reactions is stoichiometric to the amount of citrate. NADH is determined by absorbance at 340 nm.

REAGENT INFORMATION	**Barcode ID**
20 ml BUF A + Lyo A = Reagent R1	706
2 ml BUF B + Lyo B = Reagent R2	A58

Note: Labels of reagent vials have two barcodes.
For Arena analyzers, turn the short barcode to the barcode reader.
For Gallery analyzers, turn the long barcode to the barcode reader.

Concentrations

Good buffer	> 10 mmol/L
LDH	> 500 U/L
NADH	> 0.1 mmol/L
MDH	> 200 U/L
CL	> 300 U/L

Precautions
The reagents may contain sodium azide (< 0.1 %) as preservative. Do not swallow. Avoid contact with skin and mucous membranes. Take the necessary precautions for the use of laboratory reagents.

It is recommended to wash 10 ml glass vial with deionized sterile water between the reconstitutions. Washing of the vial with detergents is not recommended due to the citric acid content of some products.

Reagent Preparation
Protocol for preparing the reagents R1 and R2 for the analyzer.

Preparing reagent R1:
- Pipette the full content (appr. 20 ml) of the BUF A to Lyo A vial.
- Let it completely dissolve by mixing gently. Avoid foaming.
- Pipette the solution completely back to the original BUF A vial.
- Insert the Reagent R1 to the analyzer using the vial barcode or insert it manually without the barcode.
- When not in use, ready R1 reagent should be stored capped at 2-8°C or it can be freezed once.

Preparing reagent R2:
- Pipette 2 ml from the BUF B vial to the Lyo B vial.
- Store the BUF B vial capped at 2-8°C for further use. Protect from light. Avoid contamination at all stages.
- Let the reconstituted Lyo B vial completely dissolve by mixing gently. Avoid foaming.
- Pipette the solution completely to the clean 10 ml vial included in the kit box.
- Insert the Reagent R2 to the analyzer using the vial barcode or insert it manually without the barcode.

Note: Check that there are no bubbles on the surface of the reagent when you insert vials into the analyzer. Insert always without the vial cap.

Storage and Stability
Reagent R1 (Buffer A + Lyo A) reconstitution is stable for 10 days at 2...8 °C. It is stable for 30 days frozen once at -20 °C.

Reagent R2 (Buffer B + Lyo B) reconstitution is stable for 12 hours onboard or kept at 2...8 °C protected from light. This product is temperature sensitive (>30°C).

Reagents in unopened vials are stable at 2…8 °C until the expiry date printed on the label. Do not freeze the reagents.

Refer to the Application Notes of your analyzer for the details of on board stability of the reagents.

SAMPLES

Sample Type
Food, beverage, e.g. beer, wine, juice, and other sample material.

Other sample types may also be used. It is recommend to validate the method using spiked samples with a known amount of analyte to see the possible matrix effect of the sample.

Sample concentration and Arena/Gallery application
All method related details are in the separate application note.
Sample preparation
- In general, use colorless, clear and quite neutral liquid samples directly.
- Wine samples can be used directly.
- Turbid solutions have to be filtered or centrifuged.
- Beer and samples containing carbon dioxide have to be degassed. In this method, beer samples were degassed by adding TBP and shaking 10 min. Turbid samples were centrifuged.
- Acidic samples have to be adjusted by adding KOH /NaOH until approx. pH 8 is reached.
- Alkaline samples have to be adjusted by adding HCl until approx. pH 8 is reached.
- Strongly colored samples can also be treated with PVPP (polyvinylpolypyrrolidone, e.g. 1 g/100 ml Sample).

The Carrez-clarification should not be used due to adsorption of citric acid during sample preparation.

It is recommended to use spiked samples to validate the sample preparation step.

TEST PROCEDURE
See a separate application for the Arena or Gallery analyzer.

Manual test procedure
Wavelength 340 nm, cuvette pathlength 1 cm, reading is done against air or distilled water at 37 °C. This is an end-point reaction, 8 - 13 minutes to the end of reaction. Linearity for manual method is 20 – 400 mg/L at 37 °C as Citric acid. Sample/R1/R2 ratio is 1/40/1.

Pipette prewarmed reagents in a cuvette using the table below.

	R/B (Reagent /Blank)	**S (Sample)**
Reconstituted R1	1000 µl	1000 µl
Distilled water	25 µl	---
Sample	---	25 µl
Mix and incubate for about 3 minutes at 37 °C. Measure the absorbance AS1 and AR/B1. Then add:		
Reconstituted R2	25 µl	25 µl
Mix carefully, incubate at 37 °C and wait the end of the reaction (5-10 min). Read AS2 and AR/B2.		

Calculate for the sample AS = (AS1 – AS2).
Calculate for the Reagent/Blank AR/B = (AR/B1 - AR/B2).

Calculate the difference ΔA = AS - AR/B.

Calculation for manual method
Use this general formula to calculate the concentration:

Citric acid conc. (g/L) = V/v x 1/εd x MW/1000 x ΔAS

V = total test volume = 1.050 mL

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v = sample volume = 0.025 mL
d = pathlength = 1 cm
ε = molar coeff. NADH = 6.3 L / mmol x cm

MW = citric acid MW = 192.1

Citric acid (g/L) = 1.281 x ΔAS

Materials required but not provided
Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

Calibration
A fresh citric acid solution is used for the calibration. Weight precisely 0.050 g of pure anhydrous citric acid standard ($C_6H_8O_7$, MW = 192.124 g/mol) into a 100 ml volumetric flask and fill up to mark with distilled water. The solution has a citric acid concentration of 500 mg/l. The standard must be used fresh.

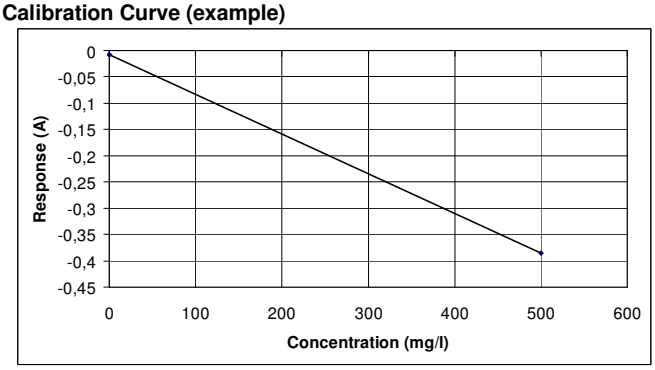
If solid monohydrous Citric Acid standard is used, weigth 1.094 times more of the standard than described above.

Quality Control
It is recommended to use quality control samples at least once an hour and after each calibration and every time a new bottle of reagent is used. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

CALCULATION OF RESULTS
The results are calculated automatically by the analyzer using a calibration curve.

Conversion factors:
mmol/l x 0.192124 = g/l
g/l x 5.2050 = mmol/l

Calibration Curve (example)



Calibrator	Response (A)	Calc. conc. (mg/l)
Water	-0.0068	0
Citric Acid std	-0.3861	500

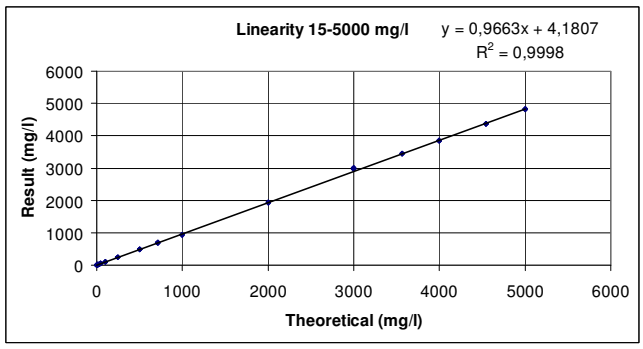
Calibration factor of this example is 1250.
Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE

Interference
The determination is specific for Citric acid.

MEASURING RANGE
The test has been developed to determine Citric acid concentrations within a measuring range from 15 to 5000 mg/l.

PERFORMANCE CHARACTERISTICS
The results obtained in individual laboratories may differ from the performance data given. Linearity testing has been performed with water based standard solutions. Different matrixes may change the linearity limits of the test.



Determination limit (=Test limit low)
The determination limit is the lowest concentration that can be measured quantitatively. The determination limit for this method is 15 mg/l.

Precision
Gallery analyzer

	Mean 61 mg/l Red wine		Mean 126 mg/l Lager beer		Mean 3779 mg/l Juice	
	SD	CV %	SD	CV %	SD	CV %
Within run	1.4	2.2	1.2	1.0	20.8	0.5
Between run	0.4	0.6	1.0	0.8	60.8	1.6
Total	1.4	2.3	1.6	1.3	64.3	1.7

A precision study was performed using Gallery for 2 days, with the number of measurements being n = 20. Arena analyzer shows similar performance.

Samples used for testing were native samples, see the table for details.

Accuracy / Method comparison
Method comparison was performed with the Gallery analyzer by comparing automated Thermo Scientific Citric acid system kit (SK) to the commercially available manual method Citric acid kit (non-SK)[4]. Compared samples included 9 randomly selected dark and lager beer and both red and white wine.

Sample	SK result (mg/l)	non-SK result (mg/l)
Beer 1	163	161
Beer 2	193	190
Beer 3	211	213
Beer 4	153	150
Beer 5	132	129
Beer 6	259	251
Beer 7	268	267
Beer 8	192	194
Beer 9	104	105
Red wine	52	55
White wine	250	253

Comparison between the automated system kit and the non-system kit method showed similar accuracy with the samples tested.

OTHER REMARKS
Note that the application performance has been verified with pure chemicals dissolved in deionized water. The results obtained in individual laboratories may differ from the given performance data due to e.g. sample matrix, concentrations or analysis environment. Each laboratory is responsible to verify the method to prove the analysis performance.

WASTE MANAGEMENT
Please refer to local legal requirements. It is recommended to empty the analyzer cuvette waste bin and waste water daily. Emptying should be done immediately after the analysis when using hazardous reagents/solutions.

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Note: If using reagents/solutions that react with each other, cuvette waste bin and waste water should be emptied and washed between use of these reagents.

BIBLIOGRAPHY

1. International Federation of Fruit Juice Producers. Code of Practice for Evaluation of Fruit and Vegetable Juices (1996) edited by Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (A.I.J.N.).
2. European Standard EN 1137. Fruit and vegetable juices: Enzymatic determination of citric acid (citrate) content by the NADH spectrometric method.
3. Brautechnische Analysenmethoden, Band III, S. 565-568 (1982), Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission (MEBAK).
4. Compendium of international methods of wine and must analysis (OIV), Edition 2007, Vol 1, MA-E-AS313-09-ACIENZ

ADDITIONAL MATERIAL

Certificate of analysis and SDS are available at
www.e-labeling.eu/TSF

Applications for Gallery and Arena automated analyzers are available upon request from the local sales representative. Information in the Application note can change without prior notice.

Manufacturer

Thermo Fisher Scientific Oy
Ratastie 2, P.O. Box 100, FI-01621 Vantaa, Finland
Tel. +358 10 329200

CONTACT INFORMATION

www.thermoscientific.com
e-mail: system.support.fi@thermofisher.com

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Changes from previous version

Reagent information
General update

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