EN

Oxalic acid

REF **984348**

❶ 2 x 18 ml Reagent R1
❷ 2 x Lyo R2
❸ 2 x Empty barcoded 20 ml reagent vial

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

METHOD
Enzymatic test with oxalate oxidase.
Method is performed at 37 °C, using 600 nm filter.

PRINCIPLE OF THE PROCEDURE
Oxalate is oxidized to carbon dioxide and hydrogen peroxide by oxalate oxidase. Hydrogen peroxide reacts in presence of peroxidase (POD) with MBTH (3-methyl-2-benzothiazolinone hydrazone) and DMAB (3-dimethylamino benzoic acid) forming a blue quinone compound. The intensity of colour is proportional to the concentration of oxalic acid in the samples and it is read at 600 nm.

REAGENT INFORMATION

		Barcode information
Reagent R1	2 x 18 ml	753
Lyo R2	2 x vial	-
Vial for R2 reconstitution		755

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

Buffer pH 3.1 ± 0.1	> 20 mmol/L
Oxalate Oxidase (Barley)	> 2 KU/L
POD	> 1000 U/L
MBTH	> 0.2 mmol/L
DMAB	> 0.9 mmol/L
Activators, Stabilizers	

Precautions
Do not swallow. Avoid contact with skin and mucous membranes. Take the necessary precautions for the use of laboratory reagents.

Preparation
Reagent R1 is ready-to-use.

Reconstitute Lyo R2 to 10 ml of deionized water. Transfer liquid to the empty barcoded R2 reagent vial.

Note: Check that there are no bubbles on the surface of the reagent when you insert vials into the analyzer.

Storage and Stability
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 on board stability of reagents.

SAMPLES

Sample Type
Food, e.g, vegetables, beverage, e.g. beer and fruit juices, and other sample material.

Sample concentration and Arena/Gallery application
All method related details are in the separate application note.

If the Arena or Gallery applications have a primary dilution of 1+9, this means that every sample is automatically first diluted with 1+9.

Sample preparation
If the sample has substances interfering the measurement, please handle it according to the following suitable preparation procedure:

Beer samples:
- Add TBP (Tributyl Phosphate) to remove foam from beer samples. Shake to remove carbon. For lager beers add activated charcoal approx. 0.3 g/10 ml sample and centrifuge to decolorize samples.

Note: The amount of activated charcoal depends on the color of the sample. Strogly colored samples may need bigger addition than lager beers.

In general:
- For beer samples or other samples containing reducing substances, activated charcoal is recommended to use to purify from interferences.
- Use colourless, clear and quite neutral liquid samples directly. Manual method is linear up to 90 mg/L, for samples with higher concentration, dilute with water.
- Turbid solutions have to be filtered or centrifuged.
- Degas samples containing carbon dioxide.
- Acidic samples should be adjusted by adding KOH /NaOH until approx. pH 4.5 – 5.5 is reached.
- For the manual method, spinach and rhubarb juices have to be diluted 1:10 with distilled water.
- Crush or homogenize solid or semi-solid samples, extract with water or dissolve in water and filter if necessary.
- Strongly coloured samples can also be treated with activated charcoal or PVPP (polyvinylpolypyrrolidone, e.g. 1 g/100 mL Sample).
- Deproteinize samples containing proteins with trichloro-acetic acid (the Carrez clarification and the perchloric acid method are not applicable to the oxalic acid test).
- Extract samples containing fat with hot water (above fat melting point). Cool on ice, sparate the fat and filter.

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

Manual test procedure
Wavelength 600 nm, cuvette pathlength 1 cm, reading against air or dist. Water at temperature 37°C. This is an end-point method with reaction time 5 minutes. Linearity is up to 180 mg/l and sample/R1/R2 ratio is 1/20/2.

Pipette prewarmed reagents in a cuvette using the table below.
For example, pipette in 3 cuvettes labelled as R/B (Blank Reagent), S (Sample and/or Controls), ST (Standard) and follow the table below.

	R/B	ST	S
Reagent R1	2000 µl	2000 µl	2000 µl
Distilled water	100 µl	----	----
Standard solution	----	100 µl	----
Sample	----	----	100 µl
Mix gently and incubate at 37°C for 5 minutes. After this one, add:			
Reconstituted Reagent R2	200 µl	200 µl	200 µl

Mix carefully and incubate at 37°C for 5 minutes, waiting the end of the reaction. Read the absorbance of the standard (Ast) and of the sample (As) against the Reagent Blank. The color is stable for at least 60 minutes.

CALCULATION for manual method:
Oxalate in mg/l = [As / Ast] x 45 (Standard conc.)

Or manual method calculation can be performed by plotting Standard solution and its dilution results to a Calibration Curve. Note that the Calibration Curve is reagent lot dependent.

For concentrations higher than 180 mg/l, dilute the sample with distilled water. Multiply the result by the dilution factor.

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

Additionally TBP (Tributyl Phosphate) is needed for beer sample pretreatment.

Activated charcoal for beer samples pretreatment.

Oxalic acid standard Cat no. 984393 (one level, water based) is not included in the kit.

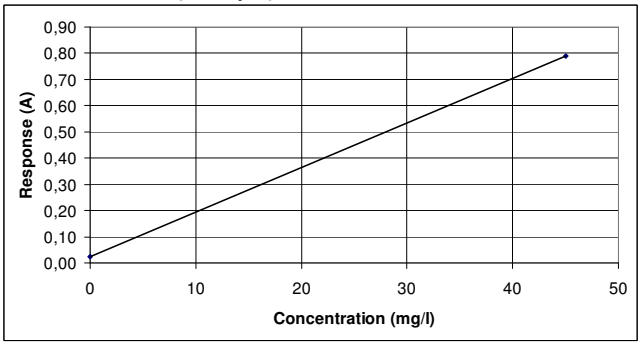
Calibration
Oxalic acid Standard Cat no 984393 can be used. Standard is ready to use.

Quality Control
Use quality control samples at least once a day 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 90.04 = mg/l
mg/l x 0.0112 = mmol/l

Calibration Curve (example)



Calibrator	Response (A)	Calc. conc. (mg/l)
Water	0.0238	0
Oxalic acid std	0.7907	45

Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE

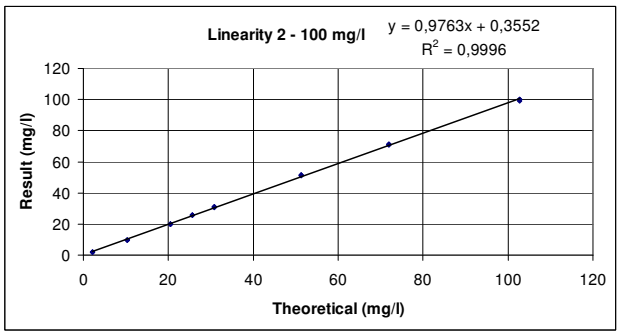
Interference/specificity
The determination is specific for oxalic acid.

Oxalic acid dihydrate (mw 126.1) may absorb moisture and therefore cause too low result when, e.g. used as QC sample.

In general, reducing substances (e.g. SO_2) or formaldehyde in the sample may interfere with the assay due to formate dehydrogenase activity decrease. Additioanally L-Ascorbic acid may interfere by formation of complexes. L-ascorbic acid can be removed by adding, e.g. 10-20 U ascorbate oxidase to sample solution.

MEASURING RANGE
The test has been developed to determine oxalic acid concentrations within a measuring range from 2 to 100 mg/l.

PERFORMANCE CHARACTERISTICS
The results obtained in individual laboratories may differ from the performance data given.



Determination limit (=Test limit low)
The determination limit is the lowest concentration that can be measured quantitatively. The determination limit for this method is 2 mg/l.

Precision

Gallery analyzer

	Mean 2 mg/l Home-brew beer		Mean 3 mg/l Lager beer		Mean 13 mg/l Dark beer	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.056	3.4	0.023	0.7	0.159	1.3
Between run	0.035	2.1	0.075	2.5	0.163	1.3
Total	0.066	4.0	0.078	2.6	0.228	1.8

A precision study was performed using Gallery, with the number of measurements being n = 40. Arena shows similar performance.

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

Accuracy / Method comparison
Accuracy of the method was tested with spiked lager beer. Seven spike levels of beer sample were analyzed with the Gallery analyzer.

Sample	Result (mg/l)	Theoretical value (mg/l)	Recovery rate (%)
Beer level 1	7.0	6.2	112
Beer level 2	7.7	8.1	95
Beer level 3	10.3	9.2	111
Beer level 4	14.5	14.1	102
Beer level 5	16.4	16.4	100
Beer level 6	25.9	28.3	92
Beer level 7	46.7	49.9	94

OTHER REMARKS
Note that the application performance has been verified with pure chemicals dissolved in deionized water and with spiked native samples. 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 bin daily. Emptying should be done immediately after the analysis when using hazardous reagents/solutions.
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 FOR THE ENZYMATIC METHOD

1. Höpner, T. & Knappe, J. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H. U. Hrsg.) 3. Aufl., Bd. 2, S. 1596-1600, Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 2nd ed., vol. 3, pp. 1551-1555, Verlag Chemie, Weinheim/Academic Press, Inc., New York and London.
2. Brautechnische Analysenmethoden, Band **111**, S. 576-580 (1982) *Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission (MEBAK),* herausgegeben von F. Drawert im Selbstverlag der MEBAK, Freising.

ADDITIONAL MATERIAL

Certificate of analysis, SDS, and 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 9 329 100, Fax +358 9 3291 0300

CONTACT INFORMATION

www.thermoscientific.com
e-mail: info.cdx.fi@thermofisher.com

Date of revision

2011-06-20

Changes from previous version

New document.