EN

Total Iron (Fe)

REF **984326**

❶	3 x 20 ml , Reagent A
❷	3 x Reagent B (Capsule)
❸	1 x 10 ml Reagent C
❹	3 x Empty 20 ml reagent vial R2

INTENDED USE

Reagent for photometric determination of Total Iron in homogenous liquid samples using automated Thermo Scientific™ Arena™ or Gallery™ analyzer.

METHOD

Colorimetric test with Ferene S.
Method is performed at 37 °C, using 600 nm filter.

PRINCIPLE OF THE PROCEDURE

Bound iron is liberated from proteins by guanidine buffer. Ascorbic acid is used to reduce the ferric iron to its ferrous state, which forms a coloured product with Ferene S. The intensity of the colour is measured at 600 nm.

Note: Ferene S method measures soluble iron and iron bound by proteins. Total Iron can be measured if all iron in the sample is dissolved.

REAGENT INFORMATION

20 ml Reag A + 1 x Reag B = R1	BARCODE ID 714
1 ml Reag C + 10 ml dH_20 = R2	BARCODE ID 718

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

Reagent A:	
Thiourea	0.1 mol/l
Guanidine buffer, pH 4.8	6 mol/l
Reagent B:	
Ascorbic acid	200 mg/capsule
Reagent C:	
Ferene S	50 mmol/l
Acetate buffer, pH 4.8	

Precautions

Reagent A is hazardous.
See separate sheet inside the kit for Hazardous- and Precautions-phrases: H302, H315, H319, P280, P301 + P312, P302 + P352, P305 + P351 + P338.
Exercise the normal precautions required for handling all laboratory reagents.
The product has to be disposed of as laboratory chemical in accordance with local regulations.

Reagent Preparation

R1: Dissolve the contents of one capsule of Reagent B into Reagent A.

Note: Check that in reagent B, the powder inside the capsule is dry and comes out easily. If not, please do not use the reagent.

R2: Pipette 1 ml of Reagent C into empty labelled 20 ml vial provided in the kit. Add 10 ml of deionized water. Cap vial and mix.

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

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

Note: Seal the plastic bag of reagent B tightly to prevent capsules to absorb moisture.

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 in the application

All method related details are in the separate application note.

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

Sample preparation

- In general, use colourless, 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.
- Strongly coloured samples can also be treated with PVPP (polyvinylpolypyrrolidone e.g. 1 g/100 mL Sample).

Carrez-clarification process cannot be used for solid, semi-solid or fat containing samples because of the method iron content.

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.

Materials required but not provided

Distilled water (aseptic and free of heavy metals; at least grade 3 as defined in ISO 3696:1987) and general laboratory equipment.

Calibration

Prepare the Calibrator solution, e.g., from AAS standard iron (1000 mg/l) from Merck. Dilute to 10 mg/l (1+99), and redilute to 1 mg/l (1+9).

Quality Control

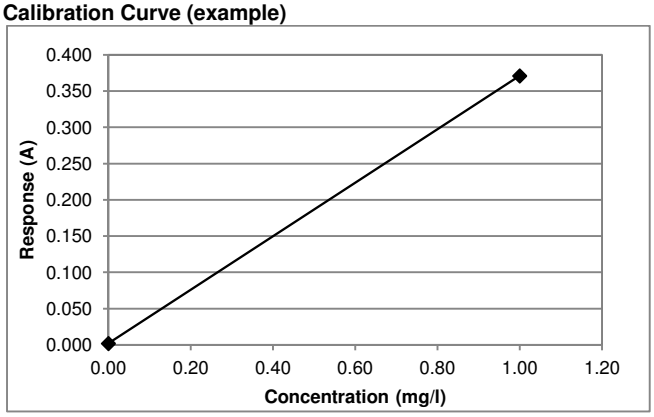
It is recommended to use quality control samples at least once a day and after each calibration and every time a new bottle of reagent is used. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the 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 55.85 = mg/l
mg/l x 0.0179 = mmol/l

Calibration Curve (example)



Note that the calibration curve is lot dependent.

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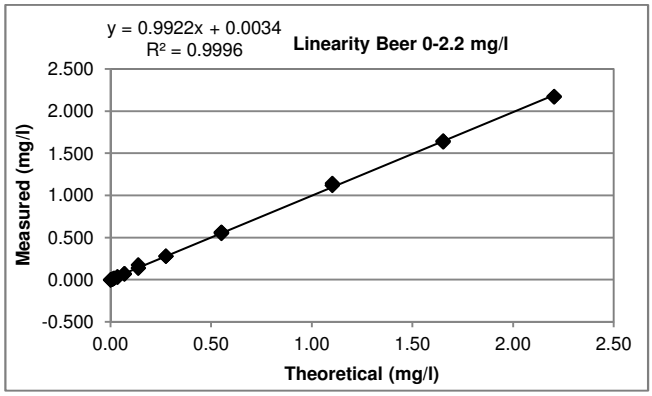
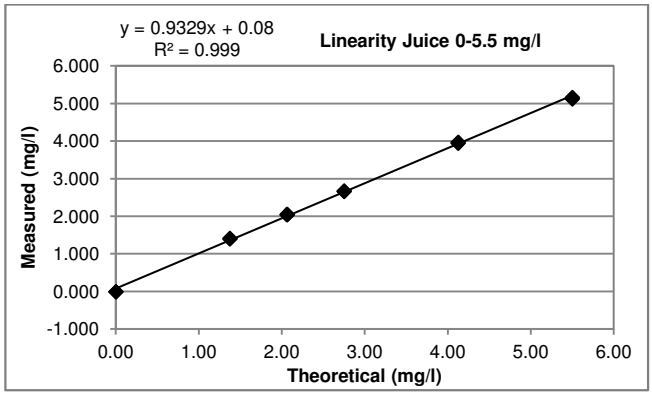
LIMITATIONS OF THE PROCEDURE

Interference
For other interfering substances, please refer to the reference 3.

MEASURING RANGE
The test has been developed to determine Total Iron concentrations within a measuring range from 0.03 to 5.50 mg/l.

PERFORMANCE CHARACTERISTICS
The results obtained in individual laboratories may differ from the performance data given. Linearity testing has been performed with spiked beer and juice 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 0.03 mg/l.

Precision
Gallery analyzer

	Dark beer (mg/)		Juice (mg/)		Red wine (mg/)	
	N	20	**N**		**N**	
	Mean	0.05	**Mean**	1.70	**Mean**	2.60
	SD	**CV %**	**SD**	**CV %**	**SD**	**CV %**
Within run	0.001	1.8 %	0.022	1.3 %	0.011	0.4 %
Between run	0.001	1.9 %	0.025	1.5 %	0.017	0.6 %
Total	0.001	2.6 %	0.033	2.0 %	0.020	0.8 %

Arena analyzer shows similar performance.

Accuracy / Method comparison
Accuracy of the method was tested with spiked native samples. Eight spike levels of beer sample and five spike levels of juice sample were analyzed.

Sample	Result (mg/l)	Theoretical value (mg/l)	Recovery rate (%)
Beer level 1	0.032	0.034	92
Beer level 2	0.068	0.069	98
Beer level 3	0.137	0.138	99
Beer level 4	0.277	0.275	101
Beer level 5	0.548	0.550	100
Beer level 6	1.122	1.101	102
Beer level 7	1.634	1.652	99
Beer level 8	2.166	2.203	98
Juice level 1	1.407	1.375	102
Juice level 2	2.045	2.063	99
Juice level 3	2.672	2.750	97
Juice level 4	3.946	4.125	96
Juice level 5	5.164	5.500	94

OTHER REMARKS
Note that the application performance has been verified with pure chemicals dissolved in deionized water and 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 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 METHOD
1. Burtis, CA and Ashwood, E R (ed.), Tietz Fundamentals of Clinical Chemistry, 5th edition, W B Saunders Company, Philadelphia, 2001, pp. 596 - 598, 992.
2. Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes; Preanalytical variables. Brochure in: Samples: From Patient to the Laboratory. GIT Verlag GmbH, Darmstadt, 1996.
3. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, Fifth Edition, AACC Press, Washington, D.C., 3-474 - 3-477, 2000.

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 preparation and storage and stablility updated.

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