

EN

# D-Isocitric Acid

REF 984322

1	3 x 16 ml Reagent 1
2	3 x 4.5 ml Reagent 2

## INTENDED USE

Reagent for photometric determination of D-Isocitric acid in homogenous liquid samples using automated Thermo Scientific™ Gallery™ or Arena™ analyzer.

## METHOD

Enzymatic test with Isocitrate-Dehydrogenase (ICDH). The bound D-Isocitric acid is determined after alkaline hydrolysis (see sample preparation).

Method is performed at 37 °C, using a 340 nm filter. 700 nm or 750 nm filter is used as a side wavelength filter.

## PRINCIPLE OF THE PROCEDURE

D-Isocitric acid + NADP → ICDH → 2-Oxoglutarate + CO<sub>2</sub> + NADPH + H<sup>+</sup>

D-Isocitric acid ester + H<sub>2</sub>O → pH 9-10 → D-Isocitric acid + Alcohol

D-Isocitric acid lactone + H<sub>2</sub>O → pH 9-10 → D-Isocitric acid

## REAGENT INFORMATION

Reagent R1	3 x 16 ml	Barcode ID 709
Reagent R2	3 x 4.5 ml	Barcode ID 710

**Note:** Labels of reagent vials have two barcodes.

For Gallery analyzers, turn the long barcode to the barcode reader.

For Arena analyzers, turn the short barcode to the barcode reader.

## Concentrations

R1	Buffer	pH 7.4
	NADP	≥ 1 mmol/l
R2	Buffer	pH 7.4
	ICDH	≥ 111 U/l

## Precautions

The reagents 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.

## Preparation

The reagents R1 and R2 are ready-to-use.

**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.

Reagents are stable 30 days on board.

## SAMPLES

### Sample Type

Homogenous food and other sample material.

### Sample concentration and Gallery/Arena application

All method related details are in the separate application note.

### Sample preparation

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

- Use clear, colorless and practically neutral liquid samples directly.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Crush or homogenize solid or semi-solid samples.
- Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water and filtrate, centrifuge or use Carrez clarification if necessary.
- Weigh sufficient quantity of fat containing samples into a volumetric flask (take care of the measuring range), extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 min. and filter. Alternatively use Carrez clarification after extraction.
- Adjust acid samples to pH 7 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min.
- Treat strongly colored samples with polyvinylpyrrolidone (PVPP e.g. 1 g/100 ml Sample).

- **Determination of acidic samples, e.g. lemon juice:**

Acidic samples with high concentration of D-Isocitric acid (e. g. lemon juice) must be diluted automatically 1+9 before analysis or adjusted to pH 7 with sodium or potassium hydroxide solution.

- **Determination of total D-Isocitric acid:**

For determination of total D-isocitric acid, bound D-Isocitric acid is released with alkaline hydrolysis. Adjust 25 ml of sample solution in a Erlenmyer flask to pH 10-11, heat up to boiling point and incubate for approximately 20 min. From time to time check pH and adjust if necessary. After cooling down to room temperature adjust pH to 7 and transfer the sample solution quantitatively into a 50 ml volumetric flask. Add 0.5 g of Polyvinylpyrrolidone (PVPP), stir for approximately 1 min and fill up to the mark with distilled water. Mix and filter.

## TEST PROCEDURE

### Automated test procedure

See a separate application for the Gallery or Arena analyzer.

### Materials required but not provided

Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

Standard solutions for calibration and quality control.

### Calibration

A fresh D-Isocitric acid solution is used for the calibration. Weight precisely 0.175 g of DL-Isocitric acid trisodium salt (C<sub>6</sub>H<sub>6</sub>O<sub>7</sub>Na<sub>3</sub>, MW = 258.07 g/mol, purity ≥ 93 %) into a 100 ml volumetric flask and fill up to mark with distilled water. The solution has a D-Isocitric acid concentration of 606 mg/l. The standard must be used fresh.

### 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 levels 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

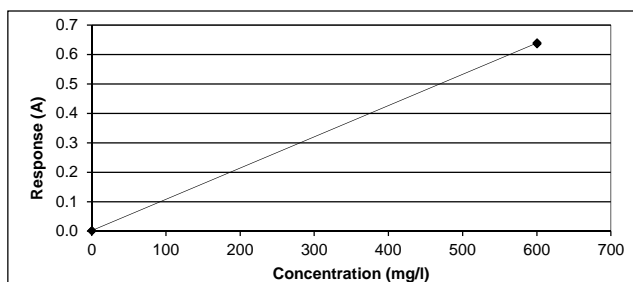
The results are calculated automatically by the analyzer using a calibration curve.

Conversion factors:

mg/l x 0.005205 = mmol/l

mmol/l x 192.12 = mg/l

### Calibration Curve (example)



Calibrator	Response (A)	Calc. conc. (mg/l)
Water	0.002	0
Isoc std	0.638	600

Calibration is measured with Gallery analyzer.

Note that the calibration curve is lot and analyzer dependent.

## LIMITATIONS OF THE PROCEDURE

### Interference

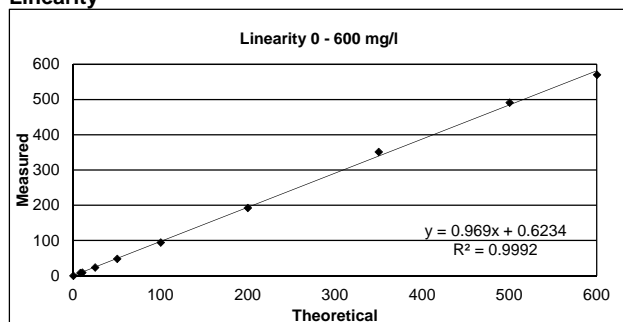
The determination is specific for D-Isocitric acid.

### MEASURING RANGE

The test has been developed to determine D-Isocitric acid concentrations within a measuring range from 10 to 600 mg/l.

**PERFORMANCE CHARACTERISTICS**

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

**Linearity**

Linearity example is measured with Gallery analyzer. Arena analyzer shows similar performance.

**Determination limit (=Test limit low)**

The determination limit is the lowest concentration that can be measured quantitatively.

The determination limit for this method is 10 mg/l.

**Precision****Gallery analyzer**

	Mean 39 mg/l		Mean 168 mg/l		Mean 554 mg/l	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.222	0.6	0.816	0.5	4.918	0.9
Between run	0.712	1.8	0.625	0.4	2.286	0.4
Total	0.745	1.9	1.028	0.6	5.424	1.0

A precision study was performed using Gallery for 5 days, with the number of measurements being n = 50.

**Arena analyzer**

	Mean 52 mg/l		Mean 167 mg/l		Mean 564 mg/l	
	SD	CV %	SD	CV %	SD	CV %
Within run	1.320	2.6	1.862	1.1	4.778	0.8
Between run	1.149	2.2	1.906	1.1	3.991	0.7
Total	1.750	3.4	2.664	1.6	6.226	1.1

A precision study was performed using Arena 20XT for 5 days, with the number of measurements being n = 50.

**OTHER REMARKS**

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.

**ADDITIONAL MATERIAL**

Certificate of analysis and SDS are available at [www.e-labeling.eu/TSF](http://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**

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[www.thermofisher.com/discreteanalysis](http://www.thermofisher.com/discreteanalysis)

**CONTACT INFORMATION**

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**Date of revision (yyyy-mm-dd)**

2018-03-23

**Changes from previous version**

Addition of sidewavelength filter. General updates.