

CITRIC ACID

UV DETERMINATION IN BIOLOGICAL FLUIDS

Kit: 5 x 20 ml
Code CI8820

SUMMARY

Citric acid is an inhibitor of the crystallization of Calcium salts. Its determination is useful to investigate the potential risk of nephrolithiasis in the patients.

PRINCIPLE

The Citric acid is changed in oxalacetate and acetate by CL (citrate lyase). This reaction is helped by a secondary one, who transforms the oxalacetate originated before (and its decarboxylated product, pyruvate) in the presence of LDH (lactate dehydrogenase), MDH (malate dehydrogenase) and NADH.

The intensity of the UV-colour at this wavelength is proportional to the concentration of Citric acid in the tested sample.

REAGENTS

Components of the kit:

Code CI8820

***REAGENT 1** (buffer, liquid, ready to use) **3 x 70 ml**

***REAGENT 2** (lyo) **5 x 20 ml**

***REAGENT 3** (starter, powder or lyo) **5 x 0.5 ml**

Good buffer > 10 mmol/L

LDH > 500 U/L

NADH > 0.1 mmol/L

MDH > 200 U/L

CL > 300 U/L

STABILITY: the reagents, stored at 2-8°C, are stable up to the expiry date shown on the package **if not contaminated during handling.**

AUXILIARY REAGENTS (Not supplied with this kit)

To assure proper test performance, we suggest following kits:

- SUBSTRATE Elevated Control (lyo) 6 x 2 mL Cod. SCE3005

- SUBSTRATE Low Control (lyo) 6 x 2 mL Cod. SCL3006

that contain a few Substrates at two different levels (see the inserts).

PREPARATION OF THE WORKING REAGENT

Dissolve a vial of *Reagent 2 with 20 ml of *Reagent 1 and mix gently till dissolution. Please avoid foaming.

A suggestion could be to aliquote in vials the quantity for each stage of analysis; to put the need for the day at 2-8°C for use, to freeze the remaining vials for next stages.

Let the reagents reach the working temperature before use.

Close immediately after handling.

Incompetent handling will release us from any responsibility.

STABILITY: the diluted *Reagent 2 is stable 10 days at 2-8°C.

Till 30 days freezed at -20°C. FREEZE only ONE TIME.

DO NOT REPEAT FREEZING.

PREPARATION OF THE WORKING STARTER

Please rem that this product is temperature sensitive (>30°C).

Dissolve a vial of *Reagent 3 with 0.5 ml of *Reagent 1 and mix gently till dissolution. Please avoid foaming.

A suggestion could be to aliquote in vials the quantity for each stage of analysis; to put the need for the day at 2-8°C for use, to freeze the remaining vials for next stages.

Let the reagents reach the working temperature before use.

Close immediately after handling.

Incompetent handling will release us from any responsibility.

STABILITY: the diluted *Reagent 3 is stable 8 hours at 2-8°C.

Till 30 days freezed at -20°C. FREEZE only ONE TIME.

DO NOT REPEAT FREEZING.

SAMPLE

- Please use limpid sample, at a NEUTRAL pH.
- If necessary, filter the sample.
- We strong suggest to neutralize the Acidic Samples at a neutral pH (max pH ≤ 8,0)

PROCEDURE

- Wavelength: 340 nm (334-365 nm)
- Pathlength: 1 cm
- Reading: against air or distilled water
- Temperature: 37°C
- Method: end-point
- Reaction: 8 - 13 minutes
- Linearity: 20 - 400 mg/L at 37°C as Citric acid
- Sample/reagents: 1/40/1

Let reagents reach the working temperature before using.

Pipette in a test tube or cuvette so labelled:

R/B: Reagent Blank, S: Sample

	R/B	S
*Working reagent	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:

*Working starter	25 µl	25 µl
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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

Use this general formula to calculate the concentration:

$$\text{Citric acid conc. (g/L)} = V/v \times 1/\epsilon d \times MW/1000 \times \Delta AS$$

V = total test volume = 1.050 ml

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

so it becomes:

$$\text{Citric acid (g/L)} = 1.281 \times \Delta AS$$

REFERENCE VALUES

Citric acid

Adults MALES 115-922 mg/24 h (0,6 - 4,8 mmol/24 h)

Adults FEMALES 250-1153 mg/24h (1,3 - 6,0 mmol/24 h)

It is suitable that every laboratory determine its reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity:

The Citric Acid Reagent is linear up to 400 mg/L (2,08 mmol/L).

For concentrations ≥ 400 mg/L, dilute the sample 1:4 with saline sol., repeat the determ. and multiply the result x 4.

Sensitivity: The minimum detectable is 4 mg/L.

**Within-run Precision:**

	Mean (mg/L) \pm 2s	CV %
Serum 1	38 \pm 0,46	1,8
Serum 2	372 \pm 4	0,9

Run-to-run (Day-to-day) Precision:

	Mean (mg/L) \pm 2s	CV %
Serum 1	39 \pm 0,66	2,1
Serum 2	374 \pm 6	1,0

Interferences: See References point 2.

Correlation: A group of 20 sera from 20 to 390 mg/L was assayed by this procedure and using a similar commercially available Citric acid Reagent. Comparison of the data gave following results:

Linear regression equation $y = 1,0037x - 0,2$
Correlation coefficient $r = 0,9976$

NOTE

1. A proportional variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. For concentrations higher than 400 mg/L dilute the sample 1:4 with saline sol., repeat the determination and multiply the result by 4.
4. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Citric acid (see References 2.).
5. PAY ATTENTION!

Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.

6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.
8. For proteic samples may be required deproteinization (spermatoc liquid for instance):

- add perchloric acid (0,33N) 2,000 mL to
the sample 0,200 mL

- mix and centrifuge 5 min x 5000 rpm
- keep 1 mL of the supernatant
- neutralize with 1 mL sodium hydroxide (NaOH 0,3 N)
- use directly this solution as sample.

- REM that the Dilution Factor will be $F = 22$; keep in mind for the Calculation

- if it will be need further dilutions, please return to Note 3).

9. The *Reagent 1 is supplied in surplus.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975).

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