CITRIC ACID

Enzymatic UV determination
on serum, urine and seminal fluid

5 x 20 ml REF **CY03-100**

PRINCIPLE

Citric acid (citrate) is converted to oxalacetate and acetate in a reaction catalyzed by the citrate lyase enzyme.
In presence of malate dehydrogenase and lactate dehydrogenase enzymes, oxalacetate and pyruvate (the product of its decarboxylation) are reduced to L-malate and L-lactate respectively, with NADH oxidation. The quantity of oxidized NADH is read at 340 nm with a spectrophotometer and is proportional to citric acid in the sample.

REAGENTS

Kit components:	REF CY03-100	Quantity
REAGENT 1 (liquid) Buffer pH 7.8	**CY03-100R1**	**1 x 105 ml**
REAGENT 2 (lyo) NADH, L-LDH, L-MDH	**CY03-100R2**	**5 vials**
REAGENT 3 (lyo) CL	**CY03-100R3**	**5 vials**
STANDARD (Std) Citric acid 250 mg/L	**CY03-100S**	**1 x 2 ml**

STABILITY: stored at 2-8°C, reagents are stable up to the expiration date on the label.

PREPARATION OF WORKING REAGENTS

REAGENT 2

Reconstitute a vial of Reagent 2 with 20 ml of Reagent 1. Shake accurately until complete dissolution.

STABILITY: 1 week at 2-8°C, 4 weeks at -20°C.
Do not freeze more than once.

REAGENT 3

Reconstitute a vial of Reagent 3 with 5 ml of distilled water. Shake gently until complete dissolution.
STABILITY:1 week at 2-8°C, 4 weeks at -20°C.
Do not freeze more than once.

SAMPLE (see note 2)

Urine.
Filter or centrifuge and use the filtrate or supernatant in the test. If sample is diluted, consider the dilution factor.
STABILITY: 1 week at 4°C, 4 weeks at -20°C.

Seminal fluid with or without deproteinization.
If seminal fluid is not deproteinized, centrifuge sample and dilute supernatant 1+9 with distilled water (dilution factor F= 10).
STABILITY: store at -20°C.

Serum with or without deproteinization.
STABILITY: 1 week at 4°C, 4 weeks at -20°C.

MANUAL ASSAY PROCEDURE

Wavelength:	340 nm
Optical path:	1 cm
Reading:	against distilled water
Temperature:	37°C
Method:	endpoint
Linearity:	up to 800 mg/L
Sample/R2/R3:	1/40/10

Pipette into test tube or cuvette labeled as it follows:

S: sample, ST: Standard, B/R: blank reagent:

	S	Std	B/R
Sample	25 µl	-	-
Standard 250 mg/L	-	25 µl	-
Distilled water			25 µl
Reagent 2	1,0 ml	1,0 ml	1,0 ml

Mix accurately and incubate at 37°C for 5 minutes. Read the solutions absorbance (A1) against distilled water for the standard (A1 std), the sample (A1 C) and the blank reagent (A1 B/R).
Add:

Reagent 3	250 µl	250 µl	250 µl

Mix accurately and incubate at 37°C for 10 minutes. Read the absorbance (A2) for the standard (A2 Std), the sample (A2 C) and the blank reagent (A2 B/R) at 340 nm against distilled water.

CALCULATION

Calculate the difference in absorbance Δ(A)= A1–A2 for the standard, the samples and the blank reagent.
The citric acid concentration in the sample is obtained by the following formula:

$$\text{Citric acid (mg/L)} = \frac{\Delta A\,(S) - \Delta A\,(B/R)}{\Delta A\,(Std) - \Delta A\,(B/R)} \times 250$$

If during the preparation the sample was diluted, multiply the result by the dilution factor F.

REFERENCE VALUES

Urine:	288 – 902 mg/ 24 hours (about 1.5 L urine)
	1.5 – 4.7 mmol 24 hours
Serum:	8.6 – 25.0 mg/L (0.045 – 0.13 mmol/L)
Seminal fluid:	4,0 – 6,9 mg/ ml (21 – 36 mmol/L)

PERFORMANCE CHARACTERISTICS

Linearity: up to 800 mg/L.
For higher values, properly dilute the samples and multiply the result by the dilution factor.

Within-run precision (on seminal liquid):

	Level 1	Level 2
Mean (mg/ml)	2,8	6,0
DS	0,022	0,080
CV %	0,78	1,33

Between-run precision (on seminal liquid):

	Level 1	Level 2
Mean (mg/ml)	3,0	6,4
DS	0,035	0,198
CV %	1,17	3,09

Interferences: lactate, pyruvate, cyclosporine A and bilirubin do not interfere. An eventual serum hemolysis does not interfere with the test.

Correlation: FAR kit to define citric acid shows a correlation coefficient of 0,96 in comparison to another kit available on the market.

NOTES

1. Refer to MSDS.
2. Sample deproteinization procedures:
   a. **Serum:**
   with perchloric acid: mix 1,0 ml of serum with 1,0 ml of iced perchloric acid (1mol/L) and centrifuge. Neutralize 1,0 ml of supernatant with 0,5 ml of potassium carbonate solution (0,3 mol/L). Incubate for 10 minutes at 0-4°C, then filter and use the filtrate in the test. Consider the dilution factor (F= 2,45) due to the sample preparation.
   by filtration: filter the serum using filters with exclusion limit of 40000 Dalton.
   b. **Seminal fluid:**
   mix 0.1 ml of sample with 1,9 ml of perchloric acid (0,3 mol/L), incubate the solution for 5-10 minutes in an iced bath and then centrifuge. Mix 1,0 ml of supernatant with 0,5 ml of potassium carbonate solution (0,73 mol/L) and incubate in an iced bath. After 15 minutes, centrifuge and use the supernatant in the test. Consider the dilution factor (F=30) due to the sample preparation.
3. Disposal of all waste material in accordance with the law.
4. Chemistry analyzer parameters are available.

REFERENCES

1. Marty et al. Clin.Chem. 30/7, 1231-1233 (1984)
2. Rossi et al. Clin. Biochemistry 30/2 143-148 (1997)

KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	caution
	read instructions for use

IVD CE

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MANUFACTURER

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