



ZINC Liquid (Nitro-PAPS)

COLORIMETRIC DETERMINATION IN BIOLOGICAL FLUIDS, WITHOUT DEPROTEINIZATION
Only for in vitro diagnostic use

Kit: 2 x 20 mL

Cod. ZIN365

SUMMARY

Zinc is present in the body with a very particular function: it is a trace element associated with a large number of enzymes (particular metalloproteins having Zn as constituent of the active site).

In the biological systems these enzymes may modulate their actions, depending from the amount of Zinc.

Biological oxido-reduction functions aren't possible with Zinc, due to its orbital electronic configuration.

Several families of enzymes have Zn involved in the active site, a few of them in critical metabolic crossroads: a lot of enzymes are anhydases, phosphatases, RNA and DNA polymerases, kinases, peptidases and dehydrogenase.

Zinc plays a great involvement in protein synthesis and gene expression: a structural and enzymatic role.

Moderate zinc deficiencies may give growth retardation in children and adolescents, mild dermatitises, hypogonadism in male, mental lethargy and impaired immune responses.

Severe cases are characterized from strong dermatitises, diarrhea, recurrent infections, weight loss, psychiatric disorders and death if not treated.

Iatrogenic conditions may happens in administration of anabolic and metal-chelating drugs, as penicillamine and corticosteroids, and synthetic diet therapies, mainly long-term treatment.

The same happens with pregnant woman, for the high uptake of zinc by the foetus; the use of oral contraceptives produces a redistribution of zinc from plasma to erythrocytes.

PRINCIPLE

Zinc dissociated from proteins, in particular conditions of ionic strength gives with chromogen Nitro-PAPS a stable colored complex, which intensity of color is proportional at the concentration of Zinc in the sample.

REAGENTS

Components of the kit:

Cod. ZIN365

***REAGENT 1** (liquid)

2 x 16 mL

Buffer pH 8.2 >0.1 mol/L

Masking agents

Stabilizers and detergents

***REAGENT 2** (liquid)

1 x 8 mL

Nitro-PAPS >0.1 mmol/L

***REAGENT 3 Standard** (liquid)

1 x 5 mL

Zinc 200 µg/dL

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

PREPARATION OF THE WORKING REAGENT

Put 4 ml of *Reagent 2 in a bottle *Reagent 1.

Mix kindly and let the reagent reach the working temperature before use. **Close immediately after handling**.

Incompetent handling will release us from any responsibility.

STABILITY: 15 days at 15-25°C (room temp.) in a dark place.

SAMPLE

- No haemolyzed serum or only heparin plasma (see Note 11.).
No EDTA-plasma!
- No haemolyzed Fresh Urine collected by standard procedure. (see Note 11.) Collect a 24-hours urine sample, measure the volume and store at 2-8°C till test, within day.
- Fresh Seminal Fluid collected by standard procedure. Centrifuge the sample at 3000 rpm for 10-15 minutes. Dilute supernatant with saline sol. 1:100, then test; consider the dilution in the calculation.

PROCEDURE

- Wavelength: 580 nm (570-600 nm)
- Pathlength: 1 cm
- Temperature: 37°C
- Method: end point
- Reaction: 5 minutes
- Linearity: up to 1000 µg/dL
- Sample/Reagent: 1/20

Let the reagents reach the working temperature before use.

Pipette in vial or cuvette so labelled :

R/B: Reagent Blank; ST: Standard; S: Sample:

	R/B	ST	S
*Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	50 µl	----	----
*Reagent 3 Standard	----	50 µl	----
Sample	----	----	50 µl

Mix carefully. Read the absorbance of the standard (Ast) and sample (As) against Reagent blank after 5 minutes at 37°C.

The color is stable for 30 minutes at room temperature.

CALCULATION

(As / Ast) x 200 = µg/dL of Zinc

(As / Ast) x 30.64 = µmol/L of Zinc

REFERENCE VALUES

Serum/Plasma	70 - 120 µg/dL (10.7 – 18.4 µmol/L)
Urine	150 – 1200 µg/L die (2.3 – 18.4 µmol/L die)
Seminal Fluid	2000 - 10000 µg/dL = 2 – 10 mg/dL (300 – 1500 µmol/L = 0.3 – 1.5 mmol/L)

It is advisable that every laboratory determines its normal 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 Zinc concentration is determined between 10-1000 µg/dL.
For concentrations ≥ 1000 µg/dL dilute the sample 1:5 with saline sol., repeat the determination and multiply the result x 5.

Sensitivity: The minimum detectable is 10 µg/dL.

Within-run Precision:

	Mean (µg/dL) ± 2s	CV %
Serum 1	103.1 ± 4.62	2.2
Serum 2	43.6 ± 8.2	9.4

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

	Mean (µg/dL) ± 2s	CV %
Serum 1	106.2 ± 8.00	3.8
Serum 2	46.6 ± 8.6	9.3

Interferences: See References point 2.

Correlation: A group of 20 samples was assayed by this procedure and using a similar commercially available Zinc Reagent on Hitachi 704. Comparison of the data gave following results:

Linear regression Y = 1.095X - 8.8

Correlation coefficient r = 0.9630n = 20

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 concentration of Zinc higher than 1000 µg/dL dilute the sample 1:5 with saline solution, repeat the determination and multiply x5.
4. PAY ATTENTION!

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

5. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Zinc (see References 2.).
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. To avoid interference or contaminations, use plastic material throwaway or very clean tubes washed with diluted HCl and distilled water.
9. Lipemic samples have to be filtered or centrifuged due to interferences in the test.
10. Bilirubin in the samples do not interfere till 20 mg/dL.
11. The zinc concentration in erythrocytes is about 10 times that of plasma, for their high concentration of carbonic anhydrase and other zinc-enzymes.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S., Effect of drugs on Clinical Lab. Test, 5th Ed. AACC Press (2000).
3. Makino T. et al., Clin. Chim. Acta, 120, 127 (1982).