

## ZINC

For in *Vitro* diagnostics use

### Colorimetric determination of Zinc in biological fluids

#### INTENDED USE

Zinc is a cofactor for more than 70 enzymes, involved in different metabolic pathways as synthesis or degradation of carbohydrates, lipids, proteins and nucleic acids. Zinc deficiency can cause anemia, hepato-splenomegalia, growth retard, retards in wounds and ulcerations cicatrization, olfaction and taste alterations. Low levels of Zinc can occur in physiological situations as pregnancy, contraceptive use, and in some pathologies such as myocardial infarction, cirrhosis, malabsorption, lung infection, lymphoma and carcinoma.

#### PRINCIPLE

Zn<sup>2+</sup> reacts with NITRO-PAPS yielding at room temperature a coloured complex which intensity is proportional to the Zinc concentration present in the sample.

The method does not require sample deproteinization either sample blank.

#### COMPOSITION

##### REAGENT A:

Borate buffer pH 8.2	370 mmol/l
Salicyaldoxime	12.5 mmol/l
Dimethylglyoxime	1.25 mmol/l
Surfactants and preservatives	

##### REAGENT B:

NITRO-PAPS	0.4 mmol/l
Preservatives	

##### STANDARD:

Nitrate zinc	1x5 ml 200 µg/dl as Zn <sup>2+</sup> ion
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#### PREPARATION OF REAGENTS

##### Bireagent procedure:

The reagents are liquids ready to use.

##### Monoreagent procedure:

Mix 4 part of Reagent A with 1 part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 5 ml of RB).

##### Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature

Working reagent is stable for 15 days at 2-8°C.

#### ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- NaCl solution (9 g/l)

#### SAMPLES

Serum, plasma with heparin, do not use haemolysed samples.

24h urine.

Seminal fluid: centrifuge for 6-10 minutes at 3000 RPM. Dilute 1:100 the supernatant with saline solution.

In biological fluids Zinc is stable 8 days at 2-8 °C.

##### Specimen collection/Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

#### INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known Zinc concentration. Check that the values obtained are within the reference range provided.

#### ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

#### Bireagent procedure:

Pipette into disposable or well clean cuvettes:

	Blank	Standard	Sample
Sample	-	-	50 µl
Distilled H <sub>2</sub> O	50 µl	-	-
Standard	-	50 µl	-
Reagent A	800 µl	800 µl	800 µl

Mix and incubate **5 minutes at room temperature** (20-25 °C). Then add:

Reagent B	200 µl	200 µl	200 µl

Mix and incubate **5 minutes at room temperature** (20-25 °C). Read the absorbance A for all cuvettes at **578 (570-582) nm** against blank.

Colour is stable for 30 minutes..

#### Monoreagent procedure:

Pipettare in cuvette a perdere o ben pulite:

	Blank	Standard	Sample
Sample	-	-	50 µl
Distilled H <sub>2</sub> O	50 µl	-	-
Standard	-	50 µl	-
Working reagent	1000 µl	1000 µl	1000 µl

Mix and incubate **5 minutes at room temperature** (20-25 °C). Read the absorbance A for all cuvettes at **578 (570-582) nm** against blank.

Colour is stable for 30 minutes.

Note: reaction volumes may be proportionally changed.

#### CALCULATION OF RESULTS

Serum, plasma:

$$\text{Zinc, } \mu\text{g/dl} = \frac{\text{A sample}}{\text{A standard}} \times 200$$

Urine (when 24h urine volume is known):

$$\text{Zinc, } \mu\text{g/24h} = \frac{\text{A sample}}{\text{A standard}} \times 2000 \times \text{l/24h}$$

Seminal fluid:

$$\text{Zinc, mg/l} = \frac{\text{A sample}}{\text{A standard}} \times 200$$

Conversion factor:

$$\text{Zinc } \mu\text{g/dl} \times 0.1530 = \text{Zinc } \mu\text{mol/l}$$

#### REFERENCE VALUES

Serum or plasma	70 ÷ 115 µg/dl
Urine (24 hours)	100 ÷ 1000 µg/24 ore
Seminal fluid	110 ÷ 1000 mg/l

Each laboratory should establish reference ranges for its own patients population.

#### ANALYTICAL PERFORMANCES

##### Precision

Intra-assay (n = 21)	Mean (µg/dl)	SD (µg/dl)	CV%
Sample	94.14	2.220	2.36

Inter-assay (n = 21)	Mean (µg/dl)	SD (µg/dl)	CV%
Sample	94.48	2.502	2.65

##### Correlation

A comparison with a commercial available products gave the following results:

Comparison on 21 samples of serum:

$$\text{Zinc LTA} = x \quad \text{Zinc competitor} = y \quad n = 21$$

$$y = 0,96262x + 4,49686 \quad r = 0,99331$$

Comparison on 21 samples of urine:

$$\text{Zinc LTA} = x \quad \text{Zinc competitor} = y \quad n = 21$$

$$y = 0,99539x + 2,9706 \quad r = 0,99984$$

Comparison on 17 samples of Seminal fluid:

$$\text{Zinc LTA} = x \quad \text{Zinc competitor} = y \quad n = 17$$

$$y = 0,96483x + 8,74142 \quad r = 0,99825$$

##### Linearity

The assay is linear up to 1000 µg/dl.

##### Sensitivity

Test sensitivity, in terms of detection limit, is 3 µg/dl.

##### Interferences

Highly lipemic sera could interfere with this method: it is recommended to centrifuge and filter the sample (0.2 µm filter).

**Do not use haemolysed samples as haemoglobin interferes.**

Bilirubin does not interfere at concentration up to 20 mg/dl.

#### PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/ECC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of the laboratory reagents according to good laboratory practice is recommended.

#### WASTE MANAGEMENT

Please refer to local legal requirements.

#### BIBLIOGRAPHY









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3. MARINGONI A., ILLUZZI R., ATB 1991 Abstract.
4. NCCLS Document, "Procedures for the collection of arterial blood specimens", Appr. Std., 3rd Ed. (1999).
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#### CONTENTS (125 tests)

Reagent A	2 x 50 ml
Reagent B	2 x 12,5 ml
Standard	1 x 5 ml
Instruction for use	1 item

#### REF. NB12100

#### SYMBOLS

-  Only for IVD use
-  Lot of manufacturing
-  Code number
-  Storage temperature interval
-  Expiration date
-  Warning, read enclosed documents
-  Read the directions
-  Biological risk

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