

ZINC

[Summary and Explanation]

Zinc is the second most abundant trace element in the body, found in more than 300 metalloenzymes. Carbonic anhydrase, alkaline phosphatase, DNA and RNA polymerases could be listed as important examples. Due to its multiple biochemical functions, zinc deficiency manifests by variety of conditions. Growth failure and stunning, alterations in immune function, diarrhea, skin lesions and alopecia are observed in case of clinical deficiency. Subclinical deficiency has effects on immune function, synthesis and action of hormones, and neurological function.

[Principle of Assay]

Zinc found in the samples change the red-orange color of 5-Br-PAPS to light pink under alkaline conditions. The change of absorbance at 548 nm is proportional to total zinc level in the sample. The assay can be calibrated with zinc sulfate dissolved in deionized water.

[Reagent Composition]

Reagent 1: Buffer Solution
Surfactants

Reagent 2: Chromogen Solution
5-Br PAPS
Surfactants
Preservatives

[Intended Use]

This product can be used for quantitative *in vitro* determination of total zinc from human serum, plasma and urine.

[Stability and Preparation of Reagents]

R1 Buffer

Contents are ready for use, no preparation is required. Stable up to the expiry date when stored at +2 to +8°C even after opened.

R2 Chromogen solution

Contents are ready for use, no preparation is required. Stable up to the expiry date when stored at +2 to +8°C even after opened.

CAL and QC Standard and levels

Contents are ready for use, no preparation is required. Stable up to the expiry date when stored at +2 to +8°C even after opened. Any Zinc calibrator is applicable, however, Rel Assay calibrator and controls are recommended. Reagents on board stability are strongly related to auto analysers cooling specification and carry-over values.

[Performance Characteristics]

Low linearity: 4 µg/dl

High linearity: 1000 µg/dl

If the results obtained were greater than linearity limit, dilute the sample 1/10 with deionized water and multiply the result by 10.

[Accuracy]

Results obtained with Rel Assay Zinc Measurement Kit and atomic absorption spectrometry, the gold standard method, are correlated.

The results obtained using 169 samples were the following:

Correlation coefficient (r): 0.98.

Regression equation: $0.97x + 2.41$

The results of the performance characteristics depend on the analyzer used.

[Precision]

Intra-assay was determined on 30 replicates on 2 controls. The %CV results were 2.32 and inter-assay %CV results were 3.54.

[Normal range]

Adult Serum: 60-120 µg/dl

Newborn Serum: 50-100 µg/dl

Urine: 300-1200 µg/24h

Each laboratory is recommended to establish their own reference values.

[Handling method]

1. This product is designed to be used in the spectrophotometer, plate reader or automated biochemistry analyzer.

2. Could be used in various automated biochemistry analyzers, an example of operating application is shown below;

Sample	R1	R2	M.wav	S.wav
14µl	160µl	30µl	548 nm	748 nm

[Components]

All reagents are ready to use

	Content	Amount
R1	Buffer	50 ml
R2	Chromogen	10 ml
Standard	262 µg/dl Zn ⁺⁺	4 ml
Level 1	50 µg/dl Zn ⁺⁺ (35-65)	4 ml
Level 2	327 µg/dl Zn ⁺⁺ (261-393)	4 ml

[Storage and Validity Date]

Storage: This kit should be stored at 2-8°C.

Validity date: The validity of each reagent is indicated on each bottle and on the kit box.

(This kit is stable for up to expiry date when stored at 2-8°C even after opened.)

[Sample]

Blood serum, plasma and urine could be used as sample.

[Procedure and Calculation]

Wavelength	548
Temperature	37 °C
Pipette into cuvette	
	Samples Standards (CAL, LEVELS, H ₂ O)
CAL, LEVELS, H ₂ O	----- 42
Sample	42 -----
Reagent 1	570 570
Reagent 2	75 75
Mix well	

Mix, read the absorbance of sample, standards, control levels and water. Absorbance obtained from water is extracted from all samples, standard and control levels' absorbance. Extracted standard absorbance is accepted as 262 µg/dl. Calculate the factor and multiply the absorbance obtained from samples with factor to calculate the result. Level 1 is expected as 33±8 µg/dl and level 2 is expected as 327±66 µg/dl.

[Interferences]

No interferences were observed with bilirubin up to 18 mg/dL, hemoglobin up to 0.5 g/dL and triglycerides up to 1000 mg/dL.

EDTA interfere with the results.

Our studies have shown that there is no interference with all other metal ions such as copper, iron, cobalt, magnesium and aluminum.

A list of drugs and other interfering substances with zinc determination has been reported by Young et. al (6).

[Recovery]

Serum pool was prepared and zinc concentration was measured. Then zinc concentrations were re-measured following addition of zinc sulfate in ratios of 1/10 and 1/100 into the same serum pool. As a result, recovery was found in between %95 and %105 range.

[Safety precautions and warning]

1. For *in vitro* diagnostic use only.
2. Do not pipette by mouth.
3. Exercise the normal precautions required for handling laboratory reagents.
4. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
5. Do not use reagents beyond the expiry date.
6. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
7. Dispose of this cleaning liquid and also such used washing cloth or tissue paper with care, as they may also contain infectious agents.
8. Health and safety data sheets are available on request.
9. Reagent Grade 1 water must be used for the assay.
10. Make sure that cuvettes of the auto-analyzer or plate reader are thoroughly cleaned before the assay to obtain accurate and sensitive results.

References

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3. Homsher R, Zak B. Spectrophotometric Investigation of Sensitive Complexing Agents for the Determination of Zinc in Serum. Clin Chem. 1985;31(8):1310-3.
4. Barnes PM, Moynahan EJ. Zinc Deficiency in Acrodermatitis Enteropathica: Multiple Dietary Ontolerance Treated with Synthetic Diet. Proc roy Soc Med. 1973;66:327-9.
5. Prasad AS. Zinc Deficiency. BMJ. 2003;326:409-10.
6. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

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