ZINC

Diagnostic reagent for determination of Zinc concentration.
Liquid. Monoreagent. Store at +15/+25°C. For in Vitro Diagnostic Use (IVD). Do not freeze.

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
At2270	141 mL	LB075	160 mL	M3N70	240 mL	TBZN70	400 mL
BY2270	350 mL	LM240	500 mL	M3N71	25 mL	TBZN71	150 mL
BY2271	280 mL	LM241	240 mL	M4N70	400 mL	TZN70	600 mL
DMZ70	333 mL	LZN70	180 mL	PL2271	240 mL	TZN71	200 mL
ER2070	120 mL	MDN70	150 mL	RC2270	44 mL	TZN72	125 mL
HN380	230 mL	MZN70	400 mL	RC2271	44 mL	ZN2270N	150 mL
KZN70	240 mL	MZN71	250 mL	RC2272	44 mL	ZN2271N	500 mL
				RD2270	225 mL	8A2270	350 mL
				S2471	150 mL	8A2271	280 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of Zinc in serum, plasma and urine.

GENERAL INFORMATION

Zinc [Zn (atomic number is 30, atomic weigt is 65,37)] is the trace element that the human body contains the most after Fe.[1] Zn ions, found in cytoplasm as 10^{-11} mol concentration and in balance with numerous Zn metalloenzymes and transcription factors, are supposed to serve as a main hormone especially regarding cell division and growth.[2]

Zn is widely distributed in food mainly bound to proteins. Bioavailability of the Zn taken via diet depends on these proteins to be digested to release Zn and allow it to bind onto the peptides, amino acides, phosphate and other ligands in the intestinal tract.[1] Zn, whose median intake is approximately 14 mg/day for men and 9 mg/day for women in the USA, is abundunt especially in red meat and fish. Wheat germ and whole bran are also good sources, however, their Zn content decreases during milling and food processing.[1,3] Zinc is absorbed in the proximal small intestine, yet its release into the plasma is controlled by metallothionine. Synthesis of this protein increases when the zinc intake is high, (limits the absorption). Metallothionine also bind copper (with a higher affinity), and as a result, high zinc intake may reduce the copper absorption in the diet.[4] Net intestinal uptake of Zn is regulated by controlling absorption efficiency versus variable dietary Zn input and ranges from 20% to 50% of dietary content. [1] Absorbed Zn is transferred via portal circulation to the liver where it will actively participate to the plasma proteins such as metalloenzymes, albumin and α2-macroglobulin.[1] Blood plasma contains less than 1% of total body Zn level and is at a narrow range of concentrations (80-120 µg/dL, 12-18 mol/L). Approximately 80% of plasma Zn is related to albumin and most of the rest is tightly bound to α2-macroglobulin.[5] Total adult body content of Zn is about 2 – 2,5 g and present in all of the tissue and organ cells that are metabolically active.

55% of the total is in the muscles and 30% is in the bones.[6,7] Prostate, semen and retina especially have high local Zn concentrations. Almost all of the Zn in erythrocytes is in carbonic anhydrous form, so RBC Zn concentration is 10 times higher than that of plasma.[1]

Fecal excretion contains both Zn in unabsorbed diet and Zn that is re-secreted to the intestines. Total amount normally equals to the total dietary intake and ranges between 10-15 mg/day in healthy populations. By conrast, urinary excretion of Zn is typically about 0,5 mg/day (7,6 µmol/day), however, it may increase prominently during a catabolic disease.[1]

Zinc is a co-factor for many enzymes like alkaline phosphatase (low plasma activity of this enzyme is an indicator of zinc deficiency), carbonate dehydratase (carbonic anhydrous), thymidine kinase and carboxypeptidase and includes a great variety of functions.[4]

Key roles of Zn in protein and nucleic acid synthesis explain growth retardation and impairment in the wound healing for individuals with Zn deficiency.[1] Large amounts of Zn (65.4 to 130.8 mg/L [1 to 2 mmol/L]s) must be secreted by the prostate in order to maintain the viability of the sperm cell and the antibacterial environment and maintain the functioning of the sperm.[8] Seminal plasma Zn concentrations are almost normal in chronic prostatitis and adenoma; however, a significant decrease (100 fold) has been recorded in Zn secretion in prostatic neoplasma.[9] Low growth rates and other developmental abnormalities can be restored by Zn supplement in children. A meta-analysis study has showed that Zn supplement has a great effect on linear growth and weight gain.[10] Patients with acrodermetitis enteropathica characterized by periorificeal and acral dermatitis, alopecia and diarrhea has abnormally low blood Zn concentrations (30 µg/dL, 4.6 µmol/L); symptoms are reversed by oral Zn supplement.[1,11] Some patients who need IV nutrition after the surgery tend to consume a considerable amount of Zn due to the poor oral intake before and after the surgery.

During a period of negative nitrogen balance, these patients may experience increased loss of Zn from the urine as a result of the catabolic process in the muscles and through the intestines caused by diarrhea.[12] Providing sufficient Zn intravenously to achieve a positive Zn balance is associated with improved nitrogen balance.[13] Zn deficiency impairs the immune system[14,15] and has a direct effect on gastrointestinal system;[6] which Zn is thought to play a role in the synthesis and effects of many hormones through Zn transcription factors.[1] Zn deficiency correlates with testosterone in the circulation system, free T4, insulin-like growth factor (IGF)-1 and low concentrations of thymulin hormones.[16,17] Severe Zn deficiency is known to affect mental health along with depression and confusion to varying degrees via Zn containing enzymes with important activity for brain development and functioning.[1,18]

TEST PRINCIPLE

Colorimetric measurement

Nitro-3′-Phosphoadenosine-5′-phosphosulfate (Nitro-PAPS) reacts with zinc in alkaline solution to form a purple colored complex. The intensity of the color complex is proportional to the zinc concentration in the sample and is measured photometrically at a wavelength of 560 nm. The interference of the measurement with copper and iron can be virtually eliminated by pH adjustment and chelating additives.

REAGENT COMPONENTS

Reagent 1:

Compositions:	
Bicarbonate Buffer	≤ 400 mmol/L
5-Br-PAPS	≤ 0.08 mmol/L
Sodium Citrate	≤ 245 mmol/L
Detergent	%1

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +15/+25˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[19]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. For plasma, trace element tubes provided by tube manufacturers should be preferred

Hemolyzed samples must not be used.

Zinc activity stability in serum and plasma:

7 days at +2/+8ºC
1 month at -20°C

Note: Plasma samples were formerly claimed to be preferred over serum due to the potential Zn contamination from erythrocytes, platelets and leukocytes during coagulation and centrifugation, yet this hasn't been confirmed clearly in the later studies.[1]

Unit Conversion:
µmol/L × 6.51 = µg/dL

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Zinc Calibrator Liquid. We recommend:

Zinc Calibrator Liquid
Ref.No: ZNC05
Ref.No: ZNC08
Ref No: ZNCRC05 (for Roche series.)

Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used. Calibration stability is 7 days.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Zinc Control Level I Liquid
Ref.No: ZNC01
Ref No: ZNCRC01 (for Roche series.)
Ref No: ZNCRC03 (for Roche series.)

Zinc Control Level II Liquid
Ref.No: ZNC02
Ref No: ZNCRC02 (for Roche series)
Ref No: ZNCRC04 (for Roche series)

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Men Serum :	72.6 – 127 µg/dL
Women Serum:	70 – 114 µg/dL
Newborn (0-30 day):	49.5 – 99.7 µg/dL
School-age children:	63.8 – 110 µg/dL
Urine:	300 - 800 µg/24h

Note 1: Zinc values may be low during menstruation and pregnancy.
Note 2: Serum/plasma Zn concentrations show both circadian and postprandial fluctuations. Concentrations

decrease after meals and are higher in the morning than in the evening.[1]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[20]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[21]

The determined analytic measuring interval for Zinc is 4-750 µg/dL.

Detection Capability

Limit of Detection (LoD): 3 µg/dL

Limit of Quantitation (LoQ): 4 µg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[22]

Linearity

This method shows measurement linearity in the activities up to 750 µg/dL.

Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For manual dilution procedure, dilute the sample 10-fold using 0.90% isotonic. After the dilution, multiply the result of rerun sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[23]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[24]

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Zinc have been given in the table 1 and 2 respectively.

Table 1. Zinc Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
95 µg/dL	1.53	1.61	80
136 µg/dL	3.47	2.55	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[25]

Table 2. Zinc Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
95 µg/dL	3.10	3.26	80
136 µg/dL	3.20	2.35	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[25]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:[26]
y= 0.98x + 5 µg/dL
r=0.98

Interference

Endogenous interferant and analyte concentrations that have been used in the Zinc scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[27,28]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Zinc interference scanning test is appropriate, is determined as ±25%.[29]

In Zinc test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Zinc Target (µg/dL)	N*	Observed Recovery %
Bilirubin 49,5 mg/dL	118,5	3	96

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted*

error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).[28]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[28]

Note: Non-hemolyzed and non-lipemic samples should be used.

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032	: Releases a very toxic gas if contacts with acid.
H317	: May cause allergic skin reaction.

Precaution

P280	: Use protective gloves / clothes / glasses / mask.
P264	: Wash your hands properly after using.
P272	: Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	: Wash with plenty of water and soap if it contacts with skin.
P333+P313	: Seek medical help if it irritates your skin or develops rash.
P362+P364	: Remove contaminated clothes and wash properly before using.

Disposal

P501	: Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests

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