Rel Assay Diagnostics www.relassay.com

MEGA TIP®
SANAYİ VE TİCARET LTD. ŞTİ.

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KFDA
Korea Food & Drug Administration

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Rel Assay Diagnostics

Research & Clinical Chemistry Assay Kits

Total Antioxidant Status Assay Kit
TAS

Total Oxidant Status Assay Kit
TOS

Oxidative Stress Index
OSI

Paraoxonase Assay Kit
PON1

Arylesterase Assay Kit
ARES

Ischemia Modified Albumin
IMA

Native Thiol Status Assay Kit
-SH NEW

Dynamic Disulfide Status
-S-S NEW

Total Thiol Status Assay Kit
-SH NEW

Zinc Assay Kit
Zn NEW

Copper Assay Kit
Cu NEW

Phosphorus (Inorganic)
P NEW

Nadp-Dependent Isocitrate Dehyrogenase
NADP-IDH NEW

Nitric Oxide
NO UD

Rel Assay Diagnostics
Total Antioxidant Status
Reagent 1
Reagent 2
Standard

Colorimetric assay kits to use at the platforms of biochemistry analyzer, spectrophotometer, microplate reader.

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TAS

3. Generation Total Antioxidant Status

TOS

Total Oxidant Status

- First and unique kit which reagents, calibrators and QC materials are liquid and stable.
- Ready to use
- Colorimetric (660 nm)
- Total Antioxidant Status Direct Measurement Kit
- Fully automated and manually measurement options
- Long lifetimes of the reagents, calibrators and QC materials
- Suitable for serum, plasma, body fluids and tissue samples
- Suitable for plants, nutrients and oil extracts
- High analytical performance charecteristics
- High precision
- High linearity
- High sensitivity

Reactive oxygen and nitrogen species are produced in metabolic and physiological processes, measurement of the combined nonenzymatic antioxidant capacity of biological fluids and other samples provides an indication of the overall capability to counteract reactive oxygen species (ROS). Under certain conditions, the increase in oxidants and decrease in antioxidants cannot be prevented, and the oxidative stress or in over 100 disorders, develops

Antioxidant molecules prevent or inhibit these harmful reactions Serum (or plasma) concentrations of different antioxidants can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive, costly, and require complicated techniques. Because the measurement measurement of different antioxidant molecules separately is not practical and their antioxidant effects are not additive, the total antioxidant capacity of a sample is measured, and this is called total antioxidant capacity (TAC), total antioxidant activity (TAA), total antioxidant power (TAOP), total antioxidant status (TAS), total antioxidant response(TAR), or other synonyms.

Serum (or plasma) concentrations of different oxidant species can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive and costly and require complicated techniques. Since the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the total oxidant status (TOS) of a sample is measured and this is named total peroxide (TP), serum oxidation activity (SOA), reactive oxygen metabolites (ROM) or some other synonyms.

Components

R1 (Buffer)	1 x 30 ml
R2 (ABTS Radical Cation)	1 x 8 ml
Standard	1 x 4 ml
QC Level 1	1 x 4 ml
QC Level 2	1 x 4 ml

TAS Test Principle

Antioxidants in the sample reduce dark blue-green colored ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent that is a vitamin E analog .

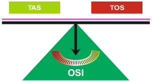
TOS Test Principle

Oxidants present in the sample oxidize the ferrous ion–chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2Equiv./L)

Generation Comparison Table

	1.Generation	2.Generation	Rel Assay®TAS 3.Generation
Reagents	Lyophilized	Liquid	**Liquid**
Reagent Life	Short	Medium	**Long (1 Year)**
Calibrators	Lyophilized	Lyophilized	**Liquid**
Calibrator Life	Short	Short	**Long (1 Year)**
QC Materials	Lyophilized	Lyophilized	**Liquid**
QC Material Life	Short	Short	**Long (1 Year)**
Tissue working	Interference	OK	**OK**
Linearity	Low	High	**High**

Oxidative Stress Index (OSI)



OSI is a new indicator for oxidative stress.
To determine OSI, TAS and TOS levels measured and the results placed in the following table.

$$\mathrm{OSI} = \frac{(\mathrm{TOS},\ \mu\mathrm{mol}\ H_2O_2\ \mathrm{equivalent/L}}{(\mathrm{TAS},\ \mu\mathrm{mol\ Trolox\ equivalent/L}} \times 100$$

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Components

R1 (Buffer)	1 x 30 ml
R2 (ABTS Radical Cation)	1 x 8 ml
Standard	1 x 4 ml
QC Level 1	1 x 4 ml
QC Level 2	1 x 4 ml

- First and unique kit which reagents and calibrators are liquid and stable.
- Ready to use
- Colorimetric (530 nm)
- Total Oxidant Status Direct Measurement Kit
- Fully automated and manually measurement options
- Long lifetimes of the reagents and calibrators
- Suitable for serum, heparinized plasma, body fluids and tissue samples
- High analytical performance charecteristics
- High precision
- High linearity
- High sensitivity
- Available of lipoprotein oxidation reaction curve

ZN

Zinc Assay Kit

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.

Princible of the 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.

- ● Sensitive and accurate
- ● Fast and convenient
- ● High reagent stability and versatility
- ● Low interference in biological samples
- ● Easy to apply simple procedure
- ● Automated, colorimetric (548nm)
- ● Measuring in variety of samples

Components

R1	1 x 30 ml
R2	1 x 8 ml
Standard	1 x 4 ml
QC Level 1	1 x 4 ml
QC Level 2	1 x 4 ml

[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.

[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.

CU

Copper Assay Kit

Copper is a very important trace element for human health due
roles in biological processes such as energy and iron metabolis
against antioxidants, synthesis of neuropeptides, and immu
functions. In case of copper deficiency, significant symptom
neutropenia, anemia and abnormalities in bone structure are
while hepatocerebral and neurodegenerative findings are seen in

Princible of the Assay

Copper found in the samples change the red-orange color of DiBr-
572 nm is proportional to total copper concentration in the sa
deionized water.

Components

R1	1 x 30 ml
R2	1 x 8 ml
Standard	1 x 4 ml
QC Level 1	1 x 4 ml
QC Level 2	1 x 4 ml

[Accuracy]

Results obtained with Rel Assay Copper Measurement Kit and atomic absorption spectrometry, the gold standard method, are correlated.
The results obtained using 173 samples were the following:
Correlation coefficient (r): 0.97.
Regression equation: 0,97x + 2,41
The results of the performance characteristics depend on the analyzer used.

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e to its key
m, defense
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s such as
observed,
toxicity..

PAESA to violet under acidic conditions. The change of absorbance at
mple. The assay can be calibrated with copper sulfate dissolved in

- Sensitive and accurate
- Fast and convenient
- High reagent stability and versatility
- Low interference in biological samples
- Easy to apply simple procedure
- Automated, colorimetric (548nm)
- Measuring in variety of samples

[Precision]

Intra-assay CV was determined with 30 replicates of 2 controls. The %CV result was1.85 and inter-assay %CV result was 2.74.

NADP-IDH

Nadp-Dependent Isocitrate Dehydrogenase

The Isocitrate Dehydrogenase Activity Assay kit provides a simple and direct procedure for measuring NADP+-dependent IDH activity in serum. The activity is determined using isocitrate as the substrate in IDH enzyme reaction. As a result of this reaction a change of absorbance at 340 nm is observed, proportional to the enzymatic activity of IDH. One unit of IDH is the amount of enzyme that will generate 1.0 µmole of NADPH per minute at pH 8.0 and 37 °C.

$$Izositrat + NAD^{+} + 2H^{+} \rightarrow 2 - oksoglutarat + NADH + H^{+} + CO_2$$

Isocitrate dehydrogenase (IDH=EC:1.1.1.42) is an enzyme that converts isocitrate to alpha-ketoglutarate (-KG) and carbondioxide(CO2). There are three isozymes of IDH (IDH1, IDH2 and IDH3), the IDH1 and IDH2 are NADP-dependent and the IDH3 utilize NAD as cofactor. NAD-dependent IDH3 is located in mitochondria and is well known for its central role for energy production in the Krebs cycle. NADP-dependent IDHs (EC 1.1.1.42) are primarily located either in cytoplasm (IDH1) or mitochondria(IDH2). Both IDH1 and IDH2 play important roles in a number of cellular metabolic functions, including glucose sensing, glutamine metabolism, lipogenesis, and regulation of cellular redox status.

IDH activity significantly increases in human serum in parenchymal diseases of liver . Although activity of mutant IDH is elevated in cancers like glioblastomas(GBM) , acute myeloid leukemia(AML) , intrahepatic cholangiocarcinoma ; particularly in the early phases of cancer, an increase is also observed in normal type of IDH enzyme activity.

P

Phosphorus (Inorganic)

Direct method for determining inorganic phosphate. Inorganic phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with yellow color. The intensity of the color formed is proportional to the inorganic phosphate concentration in the sample at 340 nm.

Components

R1 (Buffer)	1 x 30 ml
R2 (Chromogen)	1 x 30 ml
Standard 1 (3,70 mg/dl)	1 x 4 ml
Standard 2 (8,00 mg/dl)	1 x 4 ml
Level 1 QC (2,29 mg/dl)	1 x 4 ml
Level 2 QC (3,72 mg/dl)	1 x 4 ml
Level 3 QC (6,41 mg/dl)	1 x 4 ml

Most of the phosphorus in the body (80% to 85%) is calcium-bound and is found in the bones as hydroxyapatite crystals.The remainder is distributed throughout other cells of the body primarily as organic phosphorous in phospholipids and phosphoproteins. Plasma contains approximately 1% of total phosphate as inorganic phosphate, the fraction measured in routine biochemical analysis
.

Serum phosphorus may increase in hypervitaminosis D, hypoparathyroidism and renal failure. Decreased serum phosphorus is seen in rickets (vitamin D deficiency), hyperparathyroidism and Fanconi syndrome.

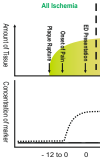
IMA

Ischemia Modified Album

- Reagents and calibrators are stable.
- Ready to use
- Colorimetric
- Fully automated and manually measurement
- Long lifetimes of the reagents
- High analytical performance charecteristics
- Precision (CV) : 0,1 %
- Linearity: IMA levels are linear
- High Sensitivity

Ischemia modified albumin (IMA) has been suggeste
marker of myocardial ischemia and has been licensed
performance of conventional biomarkers for the early

In addition to, albumin acts both as a free radical scav
radical damage to the N terminal of albumin is the ca
principle of some measurement methods for IMA



Studies in patients receiving angioplasty where ischer
rapid rise in IMA values after balloon inflation with a su
the rise in cardiac troponin and natriuretic peptides anc

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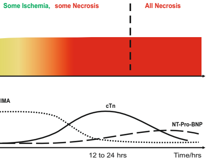
options

d as a marker for the early detection of ischemia of differing origins. IMA is a very sensitive
by the US Food and Drug Administration (FDA). IMA detection might improve the diagnostic
diagnosis of cardiac ischemia.

enger and as a chelator of transition metals, making the protein a potent antioxidant. The free
use for the reduction in the binding affinity of albumin for metals, (e.g. cobalt), which is the



mia is induced in a controlled manner, have defined the kinetics of IMA production. There is a
bsequent fall at 6 hours and return to normal by 24 hours .The rise in IMA occurs earlier than
occurs early after the onset of plaque rupture.

Thiol Disulfide Homeostasis

Native Thiol & Total Thiol Assay Kits

- Native Thiol Status (-SH)
- Dynamic Disulfide Status (-S-S-)
- Total (Oxidized and Reduced) Thiol Status (-SH + -S-S-)
- Reduced Thiol Ratio [(-SH)/ (-SH + -S-S-)]X 100
- Oxidized Thiol (disulfide) Ratio [(-S-S-)/ (-SH + -S-S-)] X 100
- Thiol Oxidation Reduction Ratio [[(-SH)/ (-S-S-)]X 100

Components of Native Thiol Assay Kit

R1	1 x 30 ml
R2	1 x 8 ml
Standard	1 x 1 ml
QC	1 x 1 ml

Components of Total Thiol Assay Kit

R1B	1 x 15 ml
R1A	1 x 1 ml
R2	1 x 30 ml
R3	1 x 8 ml
Standard	1 x 1 ml

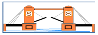
Thiols, also known as mercaptans, are a class of organic compounds that contain a sulfhydryl group (–SH) composed of a sulphur atom and a hydrogen atom attached to a carbon atom. The plasma thiol pool is mainly formed by albumin thiols, protein thiols and slightly formed by low-molecular-weight thiols such as cysteine (Cys), cysteinylglycine, glutathione, homocysteine and y- glutamylcysteine.

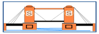
Thiols (RSH) can undergo oxidation reaction via oxidants and form disulphide (RSSR) bonds. A disulphide bond is a covalent bond; the linkage is also called a SS-bond or disulphide bridge. Under conditions of oxidative stress, the oxidation of Cys residues can lead to the reversible formation of mixed disulphides between protein thiol groups and low-molecular-mass thiols. The formed disulphide bonds can again be reduced to thiol groups; thus, dynamic thiol–disulphide homeostasis is maintained.

Dynamic thiol disulphide homeostasis status has critical roles in antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and transcription factors and cellular signalling mechanisms. Moreover, dynamic thiol disulphide homeostasis is being increasingly implicated in many disorders. There is also a growing body of evidence demonstrating that an abnormal thiol disulphide homeostasis state is involved in the pathogenesis of a variety of diseases, including diabetes [1-3], cardiovascular disease [4-10], cerebrovascular disase [11], malignacies [12], ankylosing spondylitis [13], preeclampsia [14,15], Alzheimer's disease [16], migraine [17], infections [18,19], and various disorders. Therefore, determination of dynamic thiol disulphide homeostasis can provide valuable information on various normal or abnormal biochemical processes. This easy, practical, fully automated and also optionally manual spectrophotometric assay can be used to determine plasma dynamic thiol/disulphide homeostasis.

Principle of the assays

Reducible disulphide bonds were reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native thiol groups were determined after the reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB). Half of the difference between the total thiols and the native thiols was recorded as the dynamic disulphide amount. After the native thiols (SH) and total thiols were determined, disulphide (SS) amounts, disulphide/total thiol percent ratios (SS/SH+SS), disulphide/native thiol percent ratios (SS/SH), and native thiol/total thiol percent ratios (SH/SH+SS) were calculated [20].





Application parameters of the assays

Sample volume:10 µL

R1 volume (for total –SH):10 µL

R1' volume (for native –SH):10 µL

R2 (2') volume:110 µL,

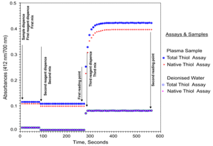
R3 (3') volume:10 µL.

Wavelength (main wavelength): 415 nm, secondary wavelength 700 nm, (optionally bichromatic).

Reading point: End-point, increasing measurement; the first absorbance is taken before the mixing of R2 and R3 and the last absorbance is taken when the reaction trace draws a plateau (assay duration is about 10 min).

Calibration type: Linear

The disulphide parameter is a value which can be calculated automatically as half of the difference of the two measured values. The assays can also be performed by manually using spectrophotometers or multiwell readers. All volumes of the samples and reagents must be increased at the same ratio. Use of a second (side) wavelength is optional.



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PON 1

Paraoxonase Assay Kit

ARES

Arylesterase Assay Kit

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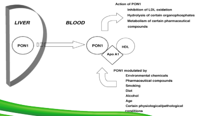
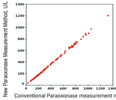
Paraoxonase-1 (PON1) is an high density lipoprotein (HDL)-associated enzyme with antioxidant and antiatherogenic functions, protecting lipoproteins against oxidative modification. It also catalyzes the hydrolysis of organophosphates such as paraoxon and aromatic carboxylic acid esters of fatty acids. It has been shown that serum paraoxonase activity decrease in diabetes mellitus, coronary artery disease, hypercholesterolaemia, iron deficiency anemia, hepatitis, cirrhosis, prostate cancer, tuberculosis and inflammations.

Fully automated paraoxonase activity measurement method consists of two different sequential reagents. The first reagent is an appropriate Tris buffer and it also contains calcium ion, which is a cofactor of PON1 enzyme. The second reagent is a new developed stabile substrate solution. The sample is mixed with the Reagent 1 and the substrate solution is added. Linear increase of the absorbance of p-nitrophenol, produced from paraoxon, is followed at kinetic measurement mode. Nonenzymatic hydrolysis of paraoxon was substracted from the total rate of hydrolysis. The molar absorptivity of p-nitrophenol is 18,290 M-1 cm-1 and one unit of paraoxonase activity is equal to 1 mol of paraoxon hydrolyzed per liter per minute at 37°C

Components	
R1 (Buffer)	1 x 30 ml
R2 (Substrate)	1 x 6 ml
Calibrator (Optional Lyophilized)	1 vial
QC Level 1 (Optional Lyophilized)	1 vial
QC Level 2 (Optional Lyophilized)	1 vial

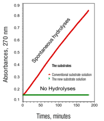
- First and unique automated kit of which reagents are liquid and stable.
- Ready to use
- Kinetic measurement
- Colorimetric (412 nm.)
- Fully automated and manual measurement options
- Long lifetimes of the reagents
- Suitable for blood serum, heparinized plasma, semen plasma, cell lysates and tissue homogenates
- High analytical performance charecteristics
- High precision
- High linearity


LIVER
BLOOD
PON1
PON1
HDL
Apo A1
Action of PON1
Inhibition of LDL oxidation
Hydrolysis of certain organophosphates
Metabolism of certain pharmaceutical compounds
PON1 modulated by
Environmental chemicals
Pharmaceutical compounds
Smoking
Diet
Alcohol
Age
Certain physiological/pathological conditions



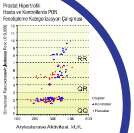
The relationship between new paraoxonase measurement method and conventional paraoxonase measurement method.

SUBSTRATE STABILITIES



PON1 192 Q/R PHENOTYPING

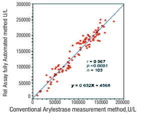
The ratio of the activity with paraoxon to the activity with phenylacetate is used to assign individuals to one of the three possible phenotypes QQ, QR, and RR. The phenotyping of PON1 by the dual substrate predicts the genotype of the enzyme with high accuracy.



Pon1 present in the sample hydrolyses phenyl acetate to its products which are phenol and acetic acid. The produced phenol is colorimetrically measured via oxidative coupling with ~~4-aminoantipyrine and~~ potassium ferricyanide. Nonenzymatic hydrolysis of phenyl acetate was substracted from the total rate of hydrolysis. The molar absorptivity of colored complex is 4000 M-1 cm-1 and one unit of arylesterase activity is equal to 1mmol of phenyl acetate hydrolyzed per liter per minute at 37°C.

Components	
R2 (Substrate)	1 x 8 ml
R3 (Chromogen)	1 x 15 ml
Diluent Solution	1 x 30 ml
Calibrator (Optional Lyophilized)	1 vial
QC Level 1 (Optional Lyophilized)	1 vial
QC Level 2 (Optional Lyophilized)	1 vial

- First and unique automated kit of which reagents are liquid and stable.
- Ready to use
- End Point Measurement
- Colorimetric
- Fully automated and manual measurement options
- Long lifetimes of the reagents
- Suitable for blood serum, heparinized plasma, semen plasma, cell lysates and tissue homogenates
- High analytical performance charecteristics
- High precision
- High linearity



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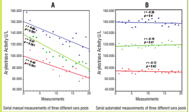
SERIAL MEASUREMENTS OF SAME SERA POOLS



Serial manuel measurements of three different sera pools

Serial automated measurements of three different sera pools