Rel Assay Diagnostics®

Clinical Chemistry Solutions

Fully Automated Arylesterase Activity Measurement Kit

MANUFACTURER

Mega Tıp San ve Tic Ltd Sti
Mücahitler Mah. 12. Cad. No:10/1
Şehitkamil/Gaziantep/TURKEY
Phone: +90 342 230 37 52

www.relassay.com
support@relassay.com

Summary

Paraoxonase-1 (PON1) is an high density lipoprotein (HDL)-associated enzyme with antioxidant and antiatherogenic functions, protecting lipoproteins against oxidative modifiicaton. 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 deficcency anemmia, hepatitis, cirrhosis, prostate cancer, tuberculosis and infammmatons.

Principle of Assay

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 subtracted from the total rate of hydrolysis. The molar absorptivity of colored complex is 4000 M -1 cm -1 and one unit of aryle sterase activity is equal to 1 mmol of phenyl acetate hydrolyzed per liter per minute at 37°C.

Storage Conditions

This kit should be stored at 4°C.

Samples

Blood serum, heparinized plasma, semen plasma, cell lysates and tissue homogenates can be used as sample.

Components

All reagents are ready to use.

Diluent Solution	30 ml
Reagent 1	Deionized Water (Any deionized water can be used)
Reagent 2	8 ml
Reagent 3	15 ml

Procedure

This assay requires predilution of sample, use the diluent which is inside of the box for this procedure.
Dilution ratio 1/100. (Sample/ Diluent)

Diluted Sample	3µL
Reagent 1	260 µL
Reagent 2	10 µL
Reagent 3	80 µL

Primary Wavel ength: 548 nm. , Secondary Wavelength 700 nm.
Method : End Point - Bichromatic

Measuring Points : First absorbance must be taken after mixing sample and R1. Last absorbance must be taken after adding and mixing R2 and R3 when the curve reaches a plateu. (Approximately 3-4 minutes after mixing)

Calibration Type : Linear
Factor : 1316

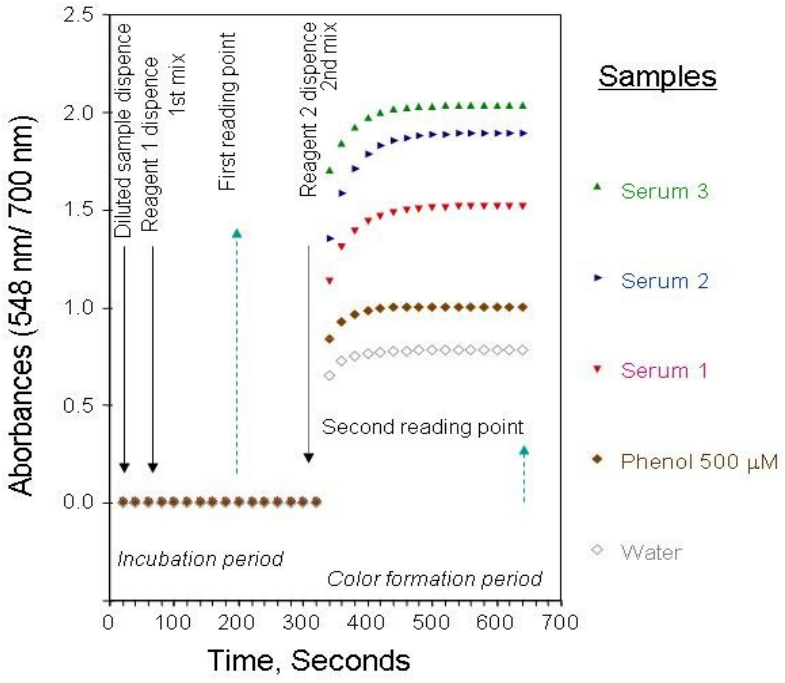
Manual measurement

In manual working, the volumes of the sample and the reagents are increased at same ratio according to the above values.

Interference and stability

Calcium chelators such as EDTA and citrate inhibited arylesterase activity. Heparin, hemolysis and bilirubin did not interfere the the assay. No signifi ant dfference was observed between fresh and non fresh serum arylesterase activities. .

Reaction kinetics of the assay



Reaction kinetics of various serum samples, phenol and water

Precision values of the assay

	Coeffificientf Varaataon, CV %
High activity sera pool	4.0
Medium activity sera pool	3.3
Low activity sera pool	3.1

References

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