Fully Automated PARAOXONASE ASSAY KIT

Product Code: RL0031

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Rel Assay Diagnostics® Clinical Chemistry Solutions

Fully Automated Paraoxonase Activity Measurement Kit

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, deficiency anemia, hepatitis, cirrhosis, prostate cancer, tuberculosis inflammations.

Principle of Assay

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 μmol of paraoxon hydrolyzed per liter per minute at 37°C

Components

All reagents are ready to use.

Reagent 1 (buffer solution) = 50 ml

Reagent 2 (substrate solution) = 5 ml

Storage Conditions

This kit should be stored at 2-8°C.

Samples

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

Procedure

The assay format of the test is given below.

Reagent 1 volume	500 μL.	
Sample volume	25 μL.	
Reagent 2 volume	25 μL.	
Wavelength	412 nm.	
Reading point	Kinetic (rate-up)	
	measurement.	
Calibration type	Factor (1294)	

Manual measurement

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

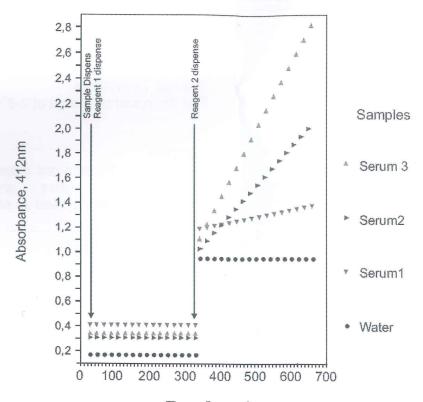
Interference and stability

Calcium chelators such as EDTA and citrate inhibited paraoxonase activity. Heparin, hemolysis and bilirubin did not interfere the the assay. Uremic plasma samples did not interfere with the assay. No significant difference was observed between fresh and non fresh serum arylesterase activities.

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Reaction kinetics of the assay



Time, Seconds

Precision values of our paraoxonase assays

	Paraoxonase Assay Coefficient of Variation, CV %	Salt Stimulated Paraoxonase Assay Coefficient of Variation, CV %
High activity sera pool	4.1	4.9
Medium activity sera pool	1.7	1.1
Low activity sera pool	1.5	1.5

References

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