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, iron defiicency annemia, heepatitis, cirrhoss, prostate cancer, tuberculosis and infla mattons.

Principle of Assay

Fully automated paraoxonase activity measurement method consists of two different seequentia reagents. The first reagent is an appropriate Tris buffer and t 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 sollutin) = 30 ml
Reagent 2 (substrate solution) = 6 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	300 μL.
Sample volume	15 μL.
Reagent 2 volume	15 μL.
Wavelength	412 nm.
Reading point	Kinetic (rate-up) measurement.
Calibration type	Factor (3445)
Unit	U/L

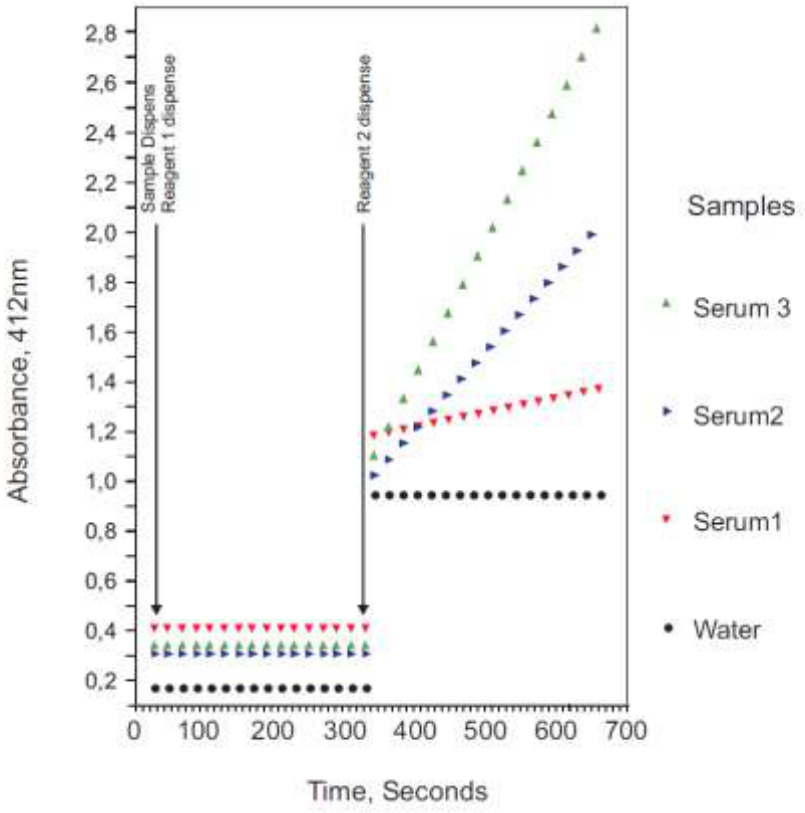
Manual measurement

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 dfference was observed between fresh and non fresh serum arylesterase activities.

Reaction kinetics of the assay



Precision values of our paraoxonase assays

	Paraoxonase Assay Coeffifici t of VaVaiation, CV %	Salt Stimulated Paraoxonase Assay Coeffifici t of VaVaiation, 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

1. Suchocka Z, Swatowska J, Pachecka J, Suchocka P; RP-HPLC determination of Paraoxonase activity in human blood serum; J of Pharmaceutical and Biomedical Analysis 2006; 42:113-119

2. Marchegiani F, Marra M, Olivieri F, Cardelli M, James RW, Boemi M, Franceschi C. Paraoxonase 1: genetics and activities during aging. Rejuvenation Res. 2008 Mar;11(1):113-27.

3. Hofer Hofer SE, Bennetts B, Chan AK, Holloway B, Karschimkus C, Jenkins AJ, Silink M, Donaghue KC. Association between PON 1 polymorphisms, PON activity and diabetes complications. J Diabetes Complications. 2006 20(5):322-8.

4. Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington PN. Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. Atherosclerosis. 1991; 86(2-3):193-9.

5. Horoz M, Aslan M, Selek S, Koylu AO, Bolukbas C, Bolukbas FF, Celik H, Erel O. PON1 status in haemodialysis patients and the impact of hepatitis C infection. Clin Biochem. 2007;40(9-10):609-14.

6. Gur M, Aslan M, Yildiz A, Demirbag R, Yilmaz R, Selek S, Erel O, Ozdogru I. Paraoxonase and arylesterase activities in coronary artery disease. Eur J Clin Invest. 2006 Nov;36(11):779-87.

7. Aslan M, Kosecik M, Horoz M, Selek S, Celik H, Erel O. Assessment of paraoxonase and arylesterase activities in patients withiron deficiency anemia. Atherosclerosis. 2007 Apr;191(2):397-402.

8. Aslan M, Nazligul Y, Horoz M, Bolukbas C, Bolukbas FF, Gur M, Celik H, Erel O. Serum paraoxonase-1 activity in Helicobacter pylori infected subjects. Atherosclerosis. 2008 Jan;19
6(1):270-4.