

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.rel assay.com
support@rel assay.com

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

Summary

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 inflammation.

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 $4000 \text{ M}^{-1} \text{ cm}^{-1}$ and one unit of arylesterase 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.

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)

<i>Diluted Sample</i>	3µL
<i>Reagent 1</i>	260 µL
<i>Reagent 2</i>	10 µL
<i>Reagent 3</i>	80 µL

Primary Wavelength: 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 plateau. (Approximately 3-4 minutes after mixing)

Calibration Type : Linear

Factor : 1316

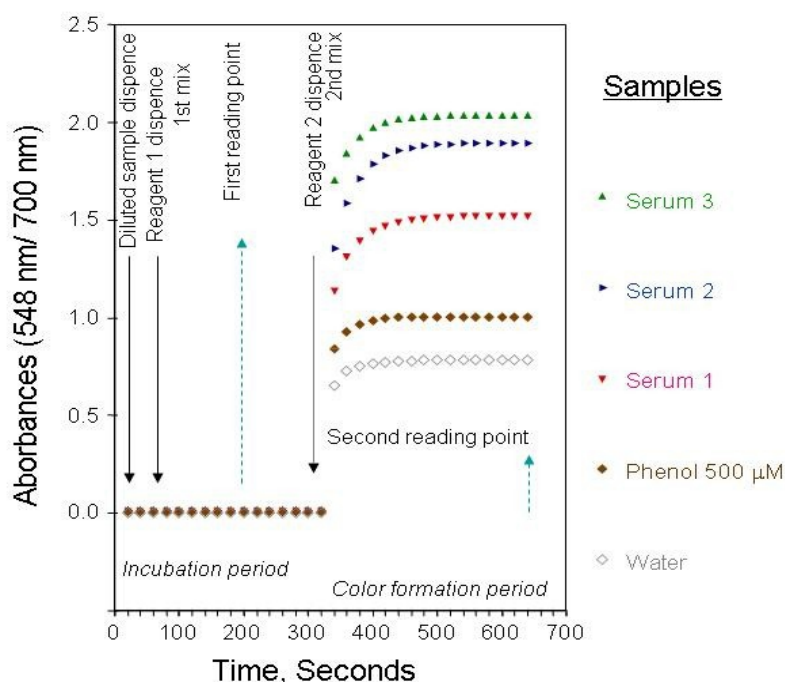
Manual measurement

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 significant difference 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

	Coefficient of Variation, CV %
High activity sera pool	4.0
Medium activity sera pool	3.3
Low activity sera pool	3.1

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 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):3228.
4. Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington P N. 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 with iron 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 activity in Helicobacter pylori infected subjects. Atherosclerosis. 2008 Jan;196(1):270-4.