PON1
Kinetic Method

Paraoxonase Activity Measurement

Summary and Explanation

Paraoxonase-1 (PON1) is a 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.

[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-1cm-1 and one unit of paraoxonase activity is equal to 1 mol of paraoxon hydrolyzed per liter per minute at 37˚C.

[Reagent Composition]

Content		Initial Concentration of Solutions
Reagent 1	**Buffer Solution**	
	TEP	%5
Reagent 2	**Substrate**	
	TEP	%5
	Paraoxon-ethyl	%0.5

[Intended Use]

This product can use for quantitative in vitro determination of paraoxonase activity from human serum and plasma.

[Stabiliy and Preparation of Reagents]

R1 *Buffer*

Contents ready for use, no need any preparation. Stable up to the expiry date when stored at +2 to +8˚C even after opening.

R2 *Substrate*

Contents ready for use, no need any preparation. Stable up to the expiry date when stored at +2 to +8˚C even after opening.

[Normal range]

Serum: 200-400 U/L

[Performance Characteristics]

1. Sensitivity

Use any de-ionized water, Absorbance under 0.04nm at 412nm.

2. Specificity

When measuring known concentration of control serum (control substance), the measured value is in ±10% range of known concentration.

3. Precision

Repeated more than 5 times the same sample to be measured, the coefficient of variation (CV)values is ±5% range.

4. AMR

Linearity 0-750(U/L)

[Handling method]

1. This product designed to be used in the spectrophotometer, plate reader or automated biochemistry analyzer.
2. To be used in various automated biochemistry analyzer, one of operating application shown as below;

R1	R2	Sample	Wavelength
500 µℓ	25 µℓ	25 µℓ	412nm

Components]

All reagents are ready to use

	Content	Amount
R1	Buffer	50ml
R2	Substrate	5ml

Mega Tıp Sanayi ve Ticaret Limited Şirketi Bahçelievler Mah. K.İsmailpaşa Sok. No:10 27010 Şahinbey Gaziantep Turkey
Telephone: +90 342 230 37 52 Fax +90 342 220 49 87 E-mail: relassay@relassay.com Web: www.relassay.com

[Storage and Validity date]

Storage: This kit should be stored at 2-8℃.

Validity date: The validity of each reagent is indicated on each bottle and on the kit.

(This kit is stable for up to expiry date when stored at 2-8℃ even after opened it.)

[Procedure]

Wavelength	412nm
Temperature	37 ˚C
Factor	1294

Pipette into cuvette	
	Samples
Sample	25 µl
Reagent 1	500 µl
Mix well	
Reagent 2	25 µl

Mix, read initial absorbance A1 after 30 seconds and start timer simultaneously. Read final absorbance A2 after 150 seconds.

[Calculation]

$\Delta Abs = [Abs \text{ at } 150^{th} \text{ Second}] - [Abs \text{ at } 30^{th} \text{ Second}]$

$$\Delta Abs/min = \frac{\Delta Abs}{2}$$

$$Results = \frac{[\Delta Abs/min] \times 22}{18290}$$

Units = U / L

[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 paraoxonase activities.

[Sample]

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

[Safety precautions and warning]

1. For In Vitro Diagnostic use only.
2. Do not pipette by mouth.
3. Exercise the normal precautions required for handling laboratory reagents.
4. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
5. Do not use reagents beyond the expiry date.
6. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
7. Dispose of this cleaning liquid and also such used washing cloth or tissue paper with care, as they may also contain infectious agents.
8. Health and safety data sheets are available on request.

References

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Mega Tıp Sanayi ve Ticaret Limited Şirketi Bahçelievler Mah. K.İsmailpaşa Sok. No:10 27010 Şahinbey Gaziantep Turkey
Telephone: +90 342 230 37 52 Fax +90 342 220 49 87 E-mail: relassay@relassay.com Web: www.relassay.com