Fully Automated PARAOXONASE ASSAY KIT

Product Code: RL0031

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**Fully Automated Paraoxonase Activity Measurement Kit**

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 stable 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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1 volume</td>
<td>500 μL</td>
</tr>
<tr>
<td>Sample volume</td>
<td>25 μL</td>
</tr>
<tr>
<td>Reagent 2 volume</td>
<td>25 μL</td>
</tr>
<tr>
<td>Wavelength</td>
<td>412 nm</td>
</tr>
<tr>
<td>Reading point</td>
<td>Kinetic (rate-up) measurement</td>
</tr>
<tr>
<td>Calibration type</td>
<td>Factor (1294)</td>
</tr>
<tr>
<td>Unit</td>
<td>U/L</td>
</tr>
</tbody>
</table>

**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.
Reaction kinetics of the assay

![Graph showing reaction kinetics]

Precision values of our paraoxonase assays

<table>
<thead>
<tr>
<th></th>
<th>Paraoxonase Assay Coefficient of Variation, CV %</th>
<th>Salt Stimulated Paraoxonase Assay Coefficient of Variation, CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>High activity sera pool</td>
<td>4.1</td>
<td>4.9</td>
</tr>
<tr>
<td>Medium activity sera pool</td>
<td>1.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Low activity sera pool</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

References