

DESCRIPTION AND INTENDED USE

Human serum arylesterase/paraoxonase (PON) is a polymorphic enzyme that catalyzes the hydrolysis of organophosphates such as paraoxon⁽¹⁾ and aromatic carboxylic acid esters of fatty acids^(2,3). Most organophosphates are neurotoxins that are widely encountered in the diet and household⁽⁴⁾.

While over the years enzymatic activity has been named with regard to the substrates required, the same enzyme has been shown to catalyze nearly all arylesterase and paraoxonase activities^(1,2,5). It is a glycoprotein containing 337 amino acid residues with a molecular weight of approximately 43 kD, and a serum concentration of about 50 mg/L^(2,5).

Atherosclerosis and its relationship to coronary heart disease has been well documented as a major cause of illness and death in the Western Hemisphere. The lipoprotein HDL, the “good” cholesterol, is inversely related to the risk of development of atherosclerosis. PON has been shown to be closely associated with HDL, and may contribute to HDL’s antiatherogenicity by preventing the oxidation of LDL, the “bad” cholesterol^(2,4,6,7). In exogenous experiments with HDL and PON, Aviram, et al, determined that both HDL-associated PON and purified PON were potent inhibitors of LDL oxidation. Their experiments implied a peroxidative role for PON due to its active hydrolysis of H₂O₂, a major reactive oxygen species (ROS) produced during oxidative stress^(7,8). Hence, the Arylesterase/Paraoxonase Assay Kit may be used in conjunction with the Total Glutathione Peroxidase Assay Kit (catalog # 0805002). Screening for total lipid peroxidation may be measured with the TBARS Assay Kit (catalog # 0801192).

In the clinical setting, PON is decreased in chronic renal failure, which may be restored to normal after transplantation⁽⁹⁾, decreased in liver cirrhosis^(3,10), and also shown to be decreased in diabetes, peripheral neuropathy, and familial hypercholesterolemia⁽¹¹⁾.

The OXltek Arylesterase/Paraoxonase Assay Kit provides a simple, reproducible method of quantifying arylesterase activity in serum or plasma.

***The Arylesterase/Paraoxonase Assay Kit is for Research Purposes Only.**








PRINCIPLE OF THE PROCEDURE

In this assay, arylesterase/paraoxonase catalyzes the cleavage of phenyl acetate resulting in phenol formation. The rate of formation of phenol is measured by monitoring the increase in absorbance at 270 nm at 25°C. The working reagent consists of 20 mM Tris/HCl buffer, pH 8.0, containing 1 mM CaCl₂ and 4 mM phenyl acetate as the substrate. Samples diluted 1:3 in buffer are added and the change in absorbance is recorded following a 20 sec lag time. One unit of arylesterase activity is equal to 1 μM of phenol formed per minute. The activity is expressed in kU/L, based on the extinction coefficient of phenol of 1310 M⁻¹cm⁻¹ at 270 nm, pH 8.0, and 25°C. Blank samples containing water are used to correct for non-enzymatic hydrolysis. A purified PON standard is included in the kit.

Arylesterase activity in matched serum and heparinized plasma samples test identical with this kit. The kit can also be used with citrated plasma, but with slightly lower results. Because calcium is required for arylesterase activity, chelating agents such as EDTA (2 mM) are unsuitable for this assay^(1,3).

The kit provides reagents sufficient for approximately 200 tests including dilution of samples.

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REAGENTS

Materials Supplied:

- Arylesterase Assay Buffer: (2-120 ml bottles/kit); contains Tris/HCl and CaCl₂
- Arylesterase Substrate: (150 µl/kit); contains phenyl acetate
- Arylesterase/Paraoxonase Standard: (300 µl/kit); contains purified PON

Handling and Storage:

Store Arylesterase Assay Buffer and Substrate at 4°C. The standard should be aliquoted and stored at -20°C or lower.

Materials required but not supplied:

- UV/vis spectrophotometer with a kinetic program.
- Water bath set to 25°C.
- Spectrophotometric quartz cuvettes.
- Adjustable pipettors with disposable pipette tips.
- Serological pipettes.
- Beaker or flask to make working solution
- Deionized distilled water (DI)

PRECAUTIONS








- Please read all instructions carefully prior to performing assay. Recommendations in the insert are to be used as guidelines.
- To avoid cross contamination, use separate pipette tips for each sample.
- Wear gloves, lab coats and safety glasses at all times.
- All contaminated materials and biohazardous material should be properly disposed and work surfaces appropriately decontaminated.
- The source for the QC Material is of human origin. All tests should be conducted using “Universal Precautions” ⁽¹²⁾.

PREPARATION OF REAGENTS

1. Working Reagent:

- Use aseptic handling to transfer 50 ml of Assay Buffer to a glass beaker. Add 25 µl of Substrate. Rinse pipette tip several times to ensure complete recovery.
- Stir vigorously for 30 min at room temperature.
- This solution can be kept at ambient temperature (24-25°C) for a period of 8-12 hours with no significant effect on assay performance. Do not freeze solution. Prepare fresh daily.

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2. Standard:

- Dilute standard 1:3 in Arylesterase Assay Buffer.
- The standard will tolerate 2-3 freeze-thaw cycles.

3. Turn on spectrophotometer, set to measure absorbance at 270 nm in the kinetic mode. Lag time is 20 sec, rate time 60 sec, read intervals 15-30 sec. If able to, set the recording range to 0.2 to 1.6 absorbance units. This will allow visualizing the reaction as it takes place.

4. Assay temperature should be set at 24-25°C either with a spectrophotometer or a water bath. Kinetic assays are very temperature-sensitive. Allow the working reagent time to warm up.

Notes:

- Samples should be run in duplicate.
- Water blanks (DI water replaces sample) should be run.
- It is recommended that each lab have serum or plasma they can run each time.

SAMPLE PREPARATION

PLASMA or SERUM

1. Collect blood using clot tube, heparin or citrate as the anticoagulant.
2. Spin down the RBC by centrifugation at 3000 rpm for 10 min at 4°C. If serum, allow to clot for 30 min before spinning.
3. Remove plasma or serum from the cells by drawing it off from the top.
4. If not used directly, freeze samples and store at -70°C until analysis.
5. Thaw out samples before analysis. Vortex well to mix.
6. Dilute samples 1:3 in Arylesterase Assay Buffer.

ASSAY PROCEDURE

Step 1: Turn on spectrophotometer and allow instrument to initialize for 15 minutes. Set instrument for kinetic parameters noted above.

Step 2: Clean cuvettes with alcohol. Rinse well with deionized water. Zero spectrophotometer at 270 nm with DI water.








Step 3: Pipette the following into the cuvette:

- 1000 µl working reagent
- 6.67 µl water, standard, or sample

Step 4: Pipette up and down to mix thoroughly, while avoiding bubble formation. Cover cuvette with parafilm and immediately invert gently 3 times.

Step 5: Place cuvette in the correct position in the spectrophotometer. Record the change in A_{270} for 1 min, following a 20 sec lag time.

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CALCULATIONS

Step 1: The net rate of increase in A_{270} for the sample can be calculated by subtracting the rate observed for the water blank from the rate observed for each sample.

Step 2: The net A_{270}/min for the test sample can be converted to μmol of phenol formed using the following relationship:

1 unit of arylesterase activity will cause the formation of 1 μmol of phenol per min at pH 8.0 at 25°C.

Molar extinction coefficient for phenol is $1310 \text{ M}^{-1} \text{ cm}^{-1} = 0.00131 \mu\text{M}^{-1} \text{ cm}^{-1}$

Step 3: Activity of arylesterase can be expressed as kilo International Unit/Liter (kU/L) of the sample or in terms of the protein content (termed the “specific activity”, U/mg protein).

Step 4: A theoretical unique factor is determined to convert change in absorbance per minute ($\Delta A/\text{min}$) to the corresponding units of enzyme activity. This factor is calculated using the following equation:

$U/L = \Delta A/\text{minute} \times F$; where F= factor

$F = (TV/SV) / .00131$ where








TV = Total Volume in μl

SV = Sample Volume in μl

0.00131 = micro molar extinction coefficient

This factor can be programmed into the spectrophotometer and the machine directly converts the change in absorbance at 270 nm ($\Delta A/\text{min}$) to activity in U/L. For this assay, with the proper volumes, the factor calculates to be $115,209.95 = 115$. Hence, activity is expressed in **kU/L**.

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EXAMPLE WITH MANUAL CALCULATION

The standard was assayed for arylesterase activity at 25°C with a cuvette path length of 1 cm using the assay procedure above. Change in absorbance was visualized on the spectrophotometer for a total of 80 sec. The first 20 sec were not taken into consideration in the calculation.

$$\text{Arylesterase Activity U/L} = 1 \mu\text{mol/min/L} = (\Delta A_{270}/\text{min}/0.00131) \times d \times (\text{TV/SV in } \mu\text{l})$$

d = 1 cm path length

blank: initial absorbance = 0.309	standard: initial absorbance = 0.599
final absorbance = 0.3104	final absorbance = 1.2223
$\Delta A/\text{min} = 0.0014$	$\Delta A/\text{min} = 0.6233$

$$\text{net rate} = 0.6233 - 0.0014 = 0.6219$$

$$\text{activity U/L} = (0.6219/0.00131) \times (1006.67/6.67) = 474.73 \times 150.93 = 71,650.999 \text{ U/L} = 71.65 \text{ kU/L}$$

or, in the shortened version, $0.6219 \times 115 = 71.52$

NOTE: Results for diluted samples must be multiplied by their dilution factor!

EXPECTED RESULTS








Table 1 shows the precision statistics of this assay for one batch of standard, in terms of percent coefficient of variation (%CV) for two lots of kits. The tests were carried out over 4 days with 10 tests performed each day in 2 batches. Arylesterase activity is expressed in terms of kU/L. The acceptable arylesterase activity range for the current lot of standard with this kit varies between a minimum value of 69.39 and a maximum value of 84.38 kU/L, with a mean of 76.89.

Precision and Reproducibility of Method

Table 1

	Intra Assay; N=10	Inter Assay; N=20	Between 4 Days; N=40	Lot to Lot variability N=80
Mean Arylesterase Activity (kU/L)	62.44	59.04	62.73	64.53
+/- SD	1.77	3.97	6.40	8.02
%CV	2.84	6.73	10.20	12.43

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Linearity and Dilutions:

The reaction has been found to be linear over a range of 10-120 kU/L for samples properly diluted from 1:2 to 1:6, with optimum results obtained with samples diluted at 1:3 as compared to samples run neat.

When visualizing reactions on the spectrophotometer, the reaction should be perfectly linear. There should be no plateauing. Excessive background is shown with jaggedness to the reaction line. These would indicate the need to either concentrate or dilute samples further.








Random Samples:

For one set of 10 serum samples diluted 1:3 in Arylesterase Assay Buffer, results ranged from 42.74 to 125.08 kU/L when corrected for the dilution. Another set ranged from 80.19 to 148.78 kU/L. It was found that serum samples are much less tolerant of freeze-thaw cycles than the standard.

Reference Ranges:

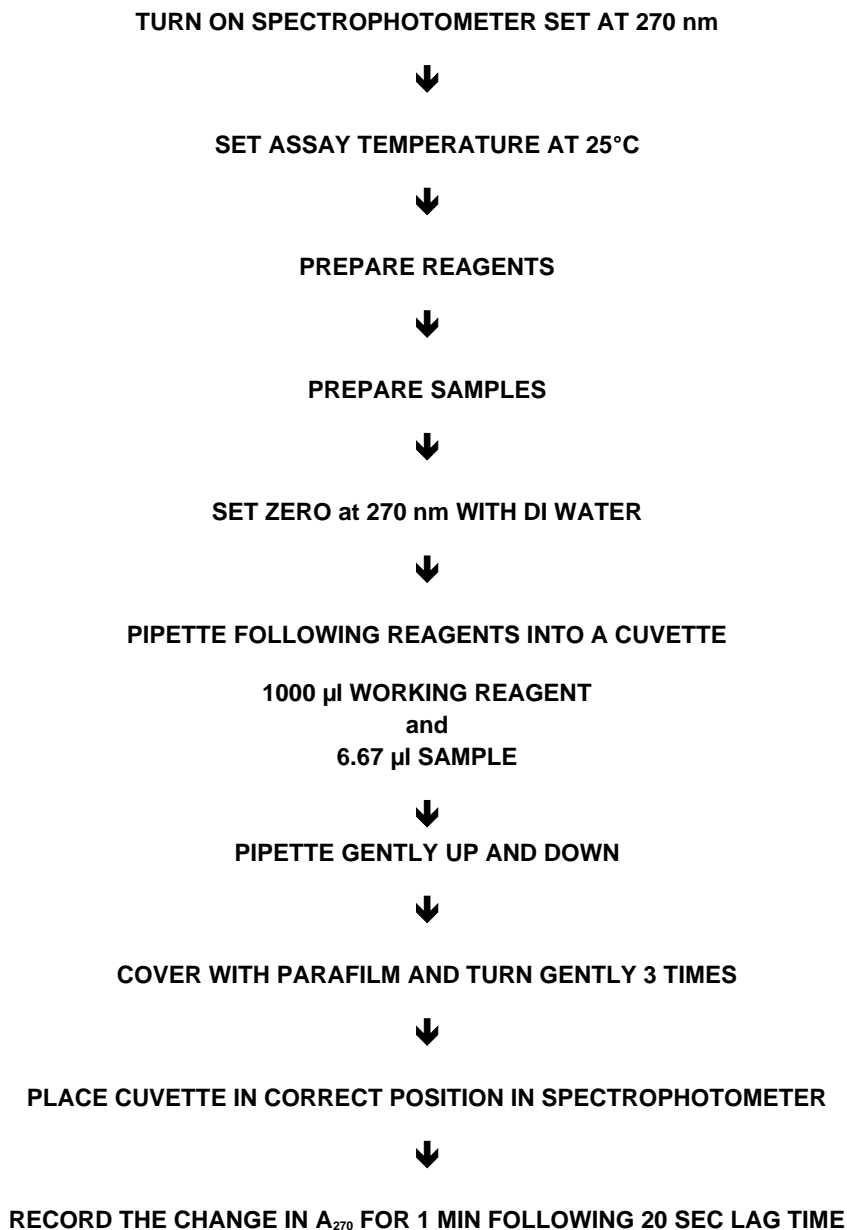
Lorentz, et al, determined a reference range for serum to be 53-186 kU/L. Urines were also included in their study, with results of 30-90 U/L ⁽³⁾.

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PROCEDURAL FLOW CHART



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