Human PON1 / paraoxonase 1 ELISA Kit

Enzyme Immunoassay for the quantification of Human PON1 / paraoxonase 1 in serum, plasma (heparin) and cell culture supernatants.

Catalog number: ARG81509

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

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INTRODUCTION

The enzyme encoded by this gene is an arylesterase that mainly hydrolyzes paroxon to produce p-nitrophenol. Paroxon is an organophosphorus anticholinesterase compound that is produced in vivo by oxidation of the insecticide parathion. Polymorphisms in this gene are a risk factor in coronary artery disease. The gene is found in a cluster of three related paraoxonase genes at 7q21.3. [provided by RefSeq, Oct 2008]

Hydrolyzes the toxic metabolites of a variety of organophosphorus insecticides. Capable of hydrolyzing a broad spectrum of organophosphate substrates and lactones, and a number of aromatic carboxylic acid esters. Mediates an enzymatic protection of low density lipoproteins against oxidative modification and the consequent series of events leading to atheroma formation. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for PON1 / paraoxonase 1 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any PON1 / paraoxonase 1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for PON1 / paraoxonase 1 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of PON1 / paraoxonase 1 bound in the initial step. The color

development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of PON1 / paraoxonase 1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

NO	Component	Quantity	Storage information
C1	Antibody-coated microplate	8 X 12 strips	4°C.
C2	Standard (Lyophilized)	2 X 2 ng/vial	4°C
C3	Standard/Sample diluent	30 ml (ready to use)	4°C
C4	Antibody conjugate concentrate (100X)	1 vial (100 µl)	4°C
C5	Antibody diluent buffer	12 ml (ready to use)	4°C
C6	HRP-Streptavidin concentrate (100X)	1 vial (100 µl)	4°C (Protect from light)
C7	HRP-Streptavidin diluent buffer	12 ml (ready to use)	4°C
C8	25X Wash buffer	20 ml	4°C
C9	TMB substrate	10 ml (ready to use)	4°C (Protect from light)
C10	STOP solution	10 ml (ready to use)	4°C
C11	Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- If crystals are observed in the 25X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.

- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

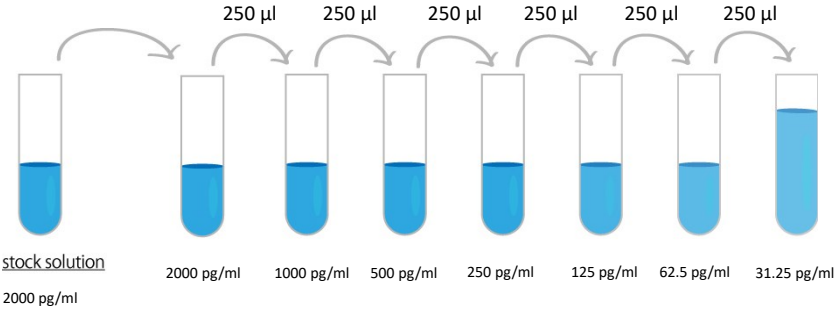
a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 25X Wash buffer (#C8) into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 25X Wash buffer + 1200 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- 1X Antibody conjugate: 20 minutes before use, dilute 100X antibody conjugate concentrate (#C4) into antibody diluent buffer (#C5) to yield 1X Detection antibody solution.
- 1X HRP-Streptavidin Solution: 20 minutes before use, dilute 100X HRP-Streptavidin concentrate solution (#C6) into HRP-Streptavidin diluent buffer (#C7) to yield 1X HRP-Streptavidin Solution buffer. Keep diluted HRP-Streptavidin Solution in dark before use.
- Sample: If the initial assay found samples contain PON1 / paraoxonase 1 higher than the highest standard, the samples can be diluted with Standard/Sample diluent (#C3) and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended to do pre-test to determine the suitable dilution factor).
- Standards: Reconstitute the standard (#C2) with 1 ml Standard/Sample diluent (#C3) to yield a stock concentration of 2000 pg/ml. Keep the buffer in the vail for at least 15 min at RT to make sure the standard is dissolved completely before making serial dilutions. The Standard/Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml. DO NOT reuse the reconstituted standard.



Dilute PON1 / paraoxonase 1 standard as according to the table below:

Standard	PON1 / paraoxonase 1 Conc.	µl of Standard diluent	µl of standard
S7	2000 pg/ml	0	1000 (2000 pg/ml Stock)
S6	1000 pg/ml	250	250 (S7)
S5	500 pg/ml	250	250 (S6)
S4	250 pg/ml	250	250 (S5)
S3	125 pg/ml	250	250 (S4)
S2	62.5 pg/ml	250	250 (S3)
S1	31.25 pg/ml	250	250 (S2)
S0	0	250	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 20 min before use.

Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls (Standard/Sample diluent) into wells, gently tap the plate to mix well. Incubate for 1.5 h at 37°C.
3. Remove the cover and discard the liquid in the wells.
4. Add 100 µl 1X Antibody conjugate into each well, gently tap the plate to mix well. Cover wells and incubate for 1 hour at 37°C.
5. Aspirate each well and wash, repeating the process two times for a total three washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 30 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl of 1X HRP-Streptavidin solution to each well, gently tap the plate to mix well. Cover wells and incubate for 30 minutes at 37°C in dark.
7. Aspirate each well and wash as step 5, but wash for 5 times.
8. Add 90 µl of TMB Reagent (#C9) to each well, gently tap the plate to mix well. Incubate for 15-25 minutes at 37°C in dark.
9. Add 100 µl of Stop Solution (#C10) to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.

10. Read the OD with a microplate reader at **450 nm immediately**. It is recommended read the absorbance within 3 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

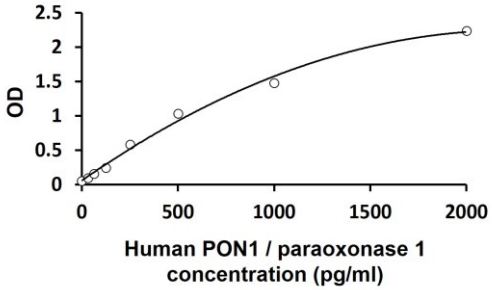
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human PON1 / paraoxonase 1 ranged from 31.2- 2000 pg/ml. The mean MDD was 15.6 pg/ml.

Specificity

This assay recognizes natural and recombinant Human PON1 / paraoxonase 1. There is no detectable cross-reactivity with other relevant proteins.

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.