HUMAN SERUM PARAOXONASE 1 (PON1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SERUM PARAOXONASE 1 CONCENTRATIONS IN SERUM.

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN SERUM PARAOXONASE 1 ELISA KIT**
Catalog No.	**SK00141-01**
Lot No.	
Formulation	**96 T**
Standard range	**1.56 - 100 ng/mL**
Sensitivity	**100 pg/mL**
Sample require	**100 µL**
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	**Serum**
Specificity	**Human Serum Paraoxonase 1**
Calibration	**Human Serum Paraoxonase 1 recombinant**
Intra-assay Precision	**4 - 6%**
Inter-assay Precision	**8 - 12%**
Storage	**2 – 8° C for 1 month, See page 2-3 for detail.**
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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DESCRIPTION

This Human Serum Paranoxonase 1 (PON1) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural PON1 from serum samples in a sandwich ELISA format.

This immunoassay contains recombinant human serum Paranoxonase 1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural serum PON1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human serum PON1. The capture antibody can bind to the human serum PON1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against PON1 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human serum PON1 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
PON1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human serum PON1.	**141-01-01**	**1 plate**
PON1 Standard – 400 ng /vial of recombinant human human serum PON1 in a buffered protein base with preservative; lyophilized.	**141-01-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of biotinylated monoclonal antibody against human serum PON1 with preservative; lyophilized.	**141-01-03**	**1 vial**
Positive Control - one vial of recombinant human PON1; lyophilized.	**141-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody & HRP Diluent Solution – 25 mL of buffered protein based solution with preservative.	**DB08A**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl solution.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate

Solution should be stored only at 2-8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

PON1 Standard - Reconstitute the PON1 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 400 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **100 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	1.0 ml	400 ng/ml
# 1	125µl of stock	375µl	100 ng/ml
# 2	250µl of 1	250µl	50 ng/ml
# 3	250µl of 2	250µl	25 ng/ml
# 4	250µl of 3	250µl	12.5 ng/ml
# 5	250µl of 4	250µl	6.25 ng/ml
# 6	250µl of 5	250µl	3.125 ng/ml
# 7	250µl of 6	250µl	1.56 ng/ml

Positive Control - Reconstitute the Positive Control with 2.0 mL of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody & HRP Diluent Solution (DB08A)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody & HRP Diluent Solution (DB08A) into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **Antibody & HRP Diluent Solution (DB08A)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light)**.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of Dilution Buffer to Blank wells.

3. Add 100 µL of each standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 20-25 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 5 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.112)
1.56	0.033
3.125	0.071
6.25	0.151
12.5	0.310
25	0.608
50	1.042
100	2.129

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Serum PON1	100
Human MPO	0

LINEARITY

To assess the linearity of the assay, pooled the research human serum samples were diluted with Dilution Buffer DB01 and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
10 x	3.309	33.09	100
20 x	1.608	32.16	97.2

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate 20-25 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 5 min.