HUMAN SOLUBLE SORTILIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE SORTILIN CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA

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.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN SOLUBLE SORTILIN ELISA**
Catalog No.	SK00472-01
Lot No.	
Formulation	96 T
Standard Range	1.56 - 200 ng/mL
Sensitivity	300 pg/mL
Sample Volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Cell Culture Supernates, Serum and Plasma
Specificity	Human sSortilin
Calibration	Human sSortilin Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble Sortilin (sSortilin) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human soluble Sortilin (sSortilin) from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human soluble Sortilin (sSortilin) and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural soluble Sortilin (sSortilin) samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human soluble Sortilin (sSortilin). The capture antibody can bind to the human soluble Sortilin (sSortilin) in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human soluble Sortilin (sSortilin) 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 soluble Sortilin (sSortilin) 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.
_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
sSortilin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against sSortilin.	**472-01-01**	**1 plate**
sSortilin Standard – refer to lot specific of recombinant sSortilin in a buffered protein base with preservative; lyophilized.	**472-01-02**	**1 vial**
Detection Antibody Concentrate – refer to lot specific concentrate of biotinylated antibody against sSortilin with preservative; lyophilized.	**472-01-03**	**1 vial**
Positive Control – one vial of recombinant sSortilin; lyophilized.	**472-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Antibody & HRP Diluent Solution – 30 mL of buffered protein based solution.	**DB01**	**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.	**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 7 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° 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 PREPARATION

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°. Avoid repeated freeze-thaw cycles.

For cell culture supernatants and samples, must use animal serum free culture medium because bovine serum cross-reacts with this kit.

Optimal dilutions should be determined by each laboratory for each application with a 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.

sSortilin Standard - Reconstitute the sSortilin standard with refer to lot specific of Dilution Buffer. 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 **200 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	Refer to lot specific	
# 1	Refer to lot specific	Refer to lot specific	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	6.25ng/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 refer to lot specific of Dilution Buffer.

Detection Antibody Concentrate – Reconstitute the Detection Antibody Concentrate with refer to lot specific of **Antibody & HRP Diluent Solution (DB01)** to prepare concentrated solution. Pipette refer to lot specific of Antibody & HRP Diluent Solution (DB01) into a 15 mL centrifuge tube and transfer the refer to lot specific of concentrated solution to the tube to make 1x working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **Antibody & HRP Diluent Solution (DB01)** 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 100µL per well of Dilution Buffer to Blank wells.
3. Add 100µL of standard dilutions in reverse order of serial dilution, samples, or positive 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 (300µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for 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 100µL 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 100µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100µL of Substrate Solution to each well. Incubate refer to lot specific on microplate shaker at room temperature. **Protect from light.**
10. Add 100µL 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 450nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the sSortilin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

Calculation of samples with a concentration exceeding that of standard 200 ng/mL may result in inaccurate, low human sSortilin levels. Such samples require further external pre-dilution according to expected human sSortilin values with Dilution Buffer in order to precisely quantify the actual human sSortilin level.

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)	CORRECTED (450NM)
Blank	0 (0.085)
0.781 (optional)	0.051
1.563	0.096
3.125	0.189
6.25	0.348
12.5	0.567
25	0.912
50	1.237
100	1.590
200	1.769

SPECIFICITY

PROTEIN NAME	CROSS-REACTIVITY
Human soluble Sortilin	100%
Human sCD36	0
Human sFNDC5	0
Human Endothelial lipase	0
Human sRAGE	0
Human VLP1	0

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 1 hour on plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate refer to lot specific on plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read at 450nm.