Human Sortilin (Soluble) ELISA KIT

Cat.No: DEIA7080

Lot. No. (See product label)

Intended Use

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 and tissue homogenates, serum and plasma in a sandwich ELISA format.

General Description

Sortilin is a protein that in humans is encoded by the SORT1 gene. The SORT1 locus shows the strongest association with serum lipoproteins of any genetic locus in the genome. In hepatocytes, sortilin participates in the presecretory degradation of nascent VLDL particles.
This gene encodes a protein that is a multi-ligand type-1 receptor with similarity to the yeast carboxypeptidase Y sorting receptor Vps10 protein. The encoded protein, a trans-Golgi network (TGN) transmembrane protein, binds a number of unrelated ligands that participate in a wide range of cellular processes; however, it lacks the typical features of a signalling receptor. In the TGN,furin mediates the activation of the mature binding form. The encoded protein consists of a large luminal domain, a single transmembrane segment and short C-terminal cytoplasmic tail. The luminal domain contains a cysteine-rich region similar to two corresponding segments in the yeast Vps10p; the cytoplasmic tail is similar to the corresponding segment of the cationindependent mannose 6-phosphate receptor and the tail also interacts with the VHS domains of GGA (Golgi-associated, gamma-adaptin homologous, ARF-interacting) proteins.
In melanocytic cells SORT1 gene expression may be regulated by MITF.

Principle Of The Test

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.

Reagents And Materials Provided

1. sSortilin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against sSortilin. 1 plate
2. sSortilin Standard - 200 ng/vial of recombinant sSortilin in a buffered protein base with preservative; lyophilized. 1 vial
3. Detection Antibody Concentrate - 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against sSortilin with preservative; lyophilized. 1 vial
4. Positive Control - one vial of recombinant sSortilin; lyophilized. 1 vial
5. Streptavidin-HRP Conjugate - 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP with preservative. 1 vial
6. Dilution Buffer - 60 mL of buffered protein based solution with preservative. 1 bottle
7. Wash Buffer - 50 mL of 10- fold concentrated buffered surfactant, with preservative. 1 bottle
8. TMB Substrate Solution - 11 mL of TMB Substrate Solution. 1 bottle
9. Stop Solution - 11 mL of 0.5M HCl. 1 bottle

Materials Required But Not Supplied

1. Microplate reader capable of absorbance measurement at 450 nm.
2. Microplate shaker (250 – 300 rpm).

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 Fax: 631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

3. Microplate washer or manifold dispenser.
4. 100 mL and 500 mL graduated cylinders.
5. Multi-channel Pipette, Pipettes and pipette tips.
6. Deionized or distilled water.

Storage

Unopened Kit: Store at 2 – 8° C for up to 8 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.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20°C or -70° C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8° C for up to 8 months (DO NOT FREEZE and PROTECT FROM LIGHT). All other components may be stored at 2 –8° C for up to 8 months.
Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

Specimen Collection And Preparation

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

Reagent Preparation

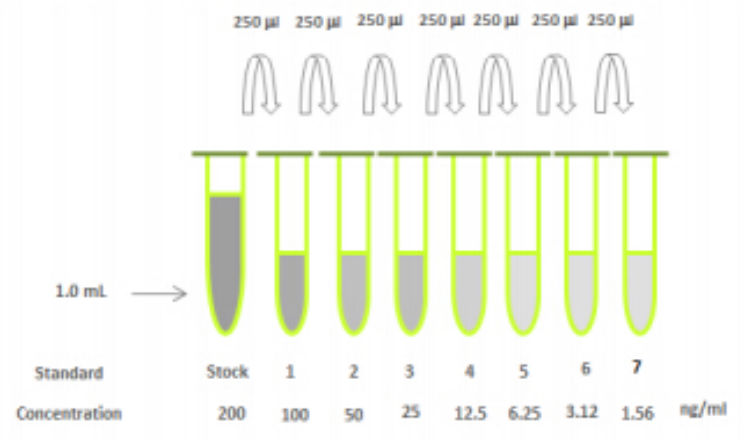
Bring all reagents to room temperature before use.

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 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 200 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 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	1.0 ml	200 ng/ml
# 1	250µl of stock	250µl	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

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 Fax: 631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com



Positive Control - Reconstitute the Positive Control with 0.5 mL of Dilution Buffer. Note: Positive Control solution could be reused within a few days if stored at -20° C or -70° C.

Detection Antibody Concentrate – Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to prepare a 10-fold concentrated solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer the 1.05 mL of 10- fold concentrated solution to the tube to make 1x working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).

Assay Procedure

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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL 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.
5. 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 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.
6. 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.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 1 hour on microplate shaker at room temperature. Protect from light.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate 1-3 minutes on microplate shaker at room temperature. Protect from light.
11. 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.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450nm.

Calculation

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 yaxis against the concentration

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 Fax: 631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

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 Standard Curve

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 (0.064)
0.78125 (optional)	0.054
1.56	0.095
3.125	0.181
6.25	0.306
12.5	0.476
25	0.788
50	1.055
100	1.360
200	1.604

Lot No.:H436810
Positive Control: 3 - 22 ng/mL

Sensitivity

The minimum detectable dose (MDD) of sSortilin was 300 pg/mL.

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

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 Fax: 631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

Reproducibility

Intra-assay Precision: 4-6%
Inter-assay Precision: 8-12%

Limitations

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
4. If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
5. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
6. This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 Fax: 631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com