HUMAN SOLUBLE CD36 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE CD36 CONCENTRATIONS IN PLASMA AND CELL CULTURE SUPERNATES

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:

ELISA NAME	HUMAN SOLUBLE CD36 ELISA
Catalog No.	SK00196-02
Lot No.	
Formulation	96 T
Standard Range	1.95 - 250 ng/mL
Sensitivity	250 pg/mL
Sample Volume	100 µL
Dilution Factor	8-16 for plasma samples **(Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	EDTA Plasma, Cell Culture Supernates
Specificity	Human sCD36
Calibration	Human sCD36 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

DESCRIPTION

This Human sCD36 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human sCD36 from cell culture supernates and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human sCD36 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural sCD36 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human sCD36. The capture antibody can bind to the human sCD36 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human sCD36 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 sCD36 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. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
sCD36 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against sCD36.	**196-02-01**	**1 plate**
sCD36 Standard – 500 ng/vial of recombinant sCD36 in a buffered protein base with preservative; lyophilized.	**196-02-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against sCD36 with preservative; lyophilized.	**196-02-03**	**1 vial**
Positive Control – one vial of recombinant human sCD36; lyophilized.	**196-02-04**	**1 vial**
Streptavidin-HRP Conjugate – 60 µL/vial, 200-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB09**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08**	**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 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 200-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.

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

Cell Culture Supernates - Remove particulates by centrifugation 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 1000 x g within 30 minutes of collection. 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.
***Note**: CD36 was expressed in platelets. Activation of platelets may increase sCD36 release. Serum samples may have high levels of sCD36.*

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

SAMPLE PREPARATION

Plasma samples may require 8 ~16 fold dilution. A suggested 8-fold dilution is 40 µL sample + 280 µL Dilution Buffer. A suggested 16-fold dilution is 20 µL sample + 300 µL Dilution Buffer.
Serum samples may require 32 ~64 fold dilution. A suggested 32-fold dilution is 10 µL sample + 310 µL Dilution Buffer. Then, to make a final dilution of 64-fold is 150 µL 32-fold diluted sample + 150 µL Dilution Buffer.
Optimal dilutions should be determined by each laboratory for each application with a pretest.
Use polypropylene test tubes.

REAGENT PREPARATION

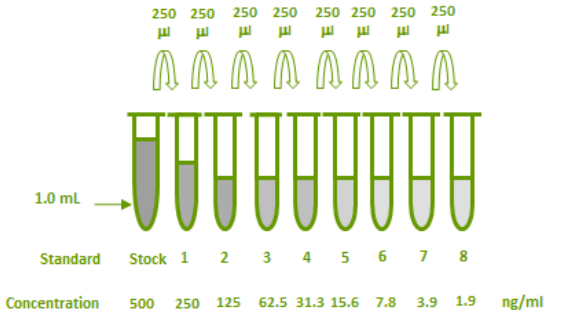
Bring all reagents to room temperature before use.

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.

Dilution Buffer (DB09) - If Dilution Buffer is highly viscous, warm in 27 - 30° C water bath until liquid flows more freely.

sCD36 Standard - Reconstitute the sCD36 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 500 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 #8. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **250 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 ml	500 ng/ml
# 1	250µl of stock	250µl	250 ng/ml
# 2	250µl of 1	250µl	125 ng/ml
# 3	250µl of 2	250µl	62.5 ng/ml
# 4	250µl of 3	250µl	31.25 ng/ml
# 5	250µl of 4	250µl	15.6 ng/ml
# 6	250µl of 5	250µl	7.8 ng/ml
# 7	250µl of 6	250µl	3.9 ng/ml
# 8	250µl of 7	250µl	1.95 ng/ml



Positive Control - Reconstitute the Positive Control with 1.0 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 11.94 mL of **HRP Diluent Solution (DB08)** into a 15 mL centrifuge tube and transfer 60 µL of 200-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)**.

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. Remove excess microplate strips from the plate frame, return them to the plastic pouch (P01) with the desiccant pack.
3. Add 100µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL of 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.
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 (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.
6. Add 100µL 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 1-10 minutes on microplate shaker at room temperature. **Protect from light.**
11. 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.
12. Determine the optical density of each well within 15 minutes, 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 sCD36 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 250 ng/ml may result in inaccurate, low human sCD36 levels. Such samples require further external pre-dilution according to expected human sCD36 values with Dilution Buffer in order to precisely quantify the actual human sCD36 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 (0.111)
1.95	0.029
3.9	0.88
7.8	0.167
15.6	0.301
31.2	0.599
62.5	0.981
125	1.439
250	2.101

- Lot No.:
- Positive Control:

SPECIFICITY

PROTEIN NAME	CROSS-REACTIVITY
Human CD36 ECD/Fc (Sf21 derived)	100%
Human CD36 ECD, His Tag	20%
Human CD320, ECD	0
Human RAGE, ECD	0
Human sLOX-1	0
Human Visfatin	0
Human FABP4	0
Human SPARC	0
Human FGF 21	0

LINEARITY

To assess the linearity of the assay, pooled human EDTA plasma samples were diluted with Dilution Buffer (DB09) and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
8 X	10.864	86.912	100
16 X	5.557	88.912	102.3
32 X	3.354	107.328	123.5
64 X	1.636	104.704	120.5

To assess the linearity of the assay, pooled human serum samples were diluted with Dilution Buffer (DB09) and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
16 X	228.022	3648.352	100
32 X	123.772	3960.704	108.6
64 X	52.938	3388.032	92.9
128 X	23.362	2990.336	82.0

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SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each 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 1-10 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 15 min.