HUMAN SOLUBLE CD36 ELISA KIT

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

PURCHASE 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	Optimal dilutions should be determined by each laboratory for each application
Sample Type	EDTA Plasma, Cell Culture Supernates
Specificity	Human Soluble CD36
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2-8°C

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

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

INTRODUCTION

Human soluble CD36 (sCD36) immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human sCD36 in cell culture supernates and plasma. It contains recombinant human sCD36 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human sCD36. Results obtained with naturally occurring sCD36 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human sCD36.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for sCD36 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sCD36 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for sCD36 is added to the wells. Following a wash to remove any unbound antibody, Streptavidin-HRP is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of sCD36 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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

_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute 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.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ 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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Human sCD36 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against sCD36.	**196-02-01**	**1 plate**
sCD36 Standard – 250 ng/vial of recombinant sCD36 in a buffered protein base with preservatives; lyophilized.	**196-02-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of biotinylated antibody against sCD36 with preservatives; 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 with preservatives	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB09**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservatives	**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 12 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 and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold Concentrate and other components may be stored at 2 - 8°C for up to 12 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate precautions should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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 plates. Activation of plates may increase sCD36 release. Serum samples may have high levels of sCD36.*

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Plasma samples may require 2 ~4 fold dilution. A suggested 2-fold dilution is 125 µL sample + 125 µL Dilution Buffer. A suggested 4-fold dilution is 62.5 µL sample + 187.5 µL Dilution Buffer.
Serum samples may require 16 ~32 fold dilution. A suggested 16-fold dilution is 20 µL sample + 310 µL Dilution Buffer. A suggested 32-fold dilution is 10 µL sample + 310 µL Dilution Buffer.

If samples with a concentration exceeding that of standard 125 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.

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

REAGENT PREPARATION

Bring all reagents to room temperature before use.

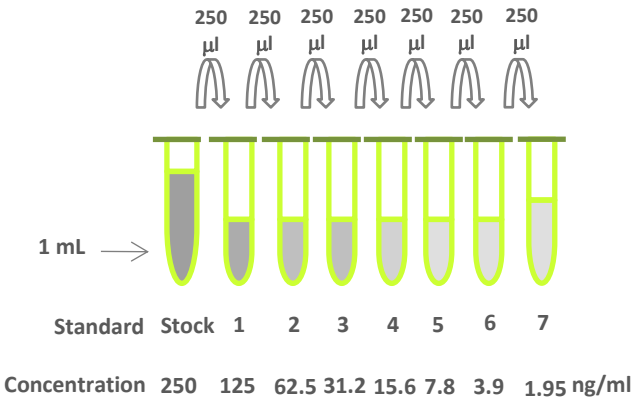
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

Dilution Buffer DB09 - If Dilution Buffer is highly viscous, warm in 27-30 ^{o} C water bath until liquid.

sCD36 Standard - Refer to vial label for reconstitution volume. Reconstitute the **sCD36** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 250 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making

dilutions. Pipette 250µL 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 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	250 ng/ml
# 1	250µl of stock	250µl	125 ng/ml
# 2	250µl of 1	250µl	62.5 ng/ml
# 3	250µl of 2	250µl	31.25 ng/ml
# 4	250µl of 3	250µl	15.6 ng/ml
# 5	250µl of 4	250µl	7.8 ng/ml
# 6	250µl of 5	250µl	3.9 ng/ml
# 7	250µl of 6	250µl	1.95 ng/ml



Detection Antibody Concentrate – Reconstitute the Detection Antibody Concentrate with 105 µL Dilution Buffer to prepare 100-fold Concentrate. Pipette 10.395 mL of Dilution Buffer into a 15mL centrifuge tube and transfer the 105 µL 100-fold Detection Antibody Concentrate to the tube to make working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB08)** 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.

Positive Control - Reconstitute the **Positive Control** with 1 mL of Dilution Buffer.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

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 and seal.
3. Add 100µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 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 Concentrate working solution to each well. Cover with 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 40 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100µL of Substrate Solution to each well. Incubate 12-17 minutes 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 125 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.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human CD36, ECD/Fc.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of sCD36 was 250 pg/mL.

TYPICAL DATA

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

STANDARD (NG/ML)	CORRECTED (450NM)
Blank	0 (0.104)
1.95	0.068
3.9	0.143
7.8	0.273
15.6	0.462
31.2	0.872
62.5	0.989
125	1.306
250	1.423

SPECIFICITY

This assay recognizes both natural and recombinant human Soluble CD36. The factors listed below were prepared at 25µg/mL in Dilution Buffer, and assayed for cross reactivity.

PROTEIN NAME	CROSS-REACTIVITY
Human CD36 ECD	100%
Human CD36 ECD (E. Coli derived)	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

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody Concentrate 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 40 minutes on plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 12-17 min on plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 15 min