HUMAN SOLUBLE CD36 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE CD36 CONCENTRATIONS IN SERUM, 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:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN SOLUBLE CD36 ELISA**
Catalog No.	SK00196-02
Lot No.	
Formulation	96 T
Standard Range	1 – 62.5 ng/mL
Sensitivity	100 pg/mL
Sample Volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	EDTA Plasma, Serum, Cell Culture Supernates
Specificity	Human sCD36
Calibration	Human CD36 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C up to 1 month, see page 2 -3 for more information
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble CD36 (sCD36) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human soluble CD36 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human soluble CD36 (extracellular domain) and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural soluble CD36 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human soluble CD36. The capture antibody can bind to the human soluble CD36 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human soluble CD36 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 CD36 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

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_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.

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 – refer to lot of recombinant sCD36 in a buffered protein base with preservative; lyophilized.	**196-02-02**	**1 vial**
Detection Antibody Concentrate – refer to lot 10-fold concentrate of biotinylated antibody against sCD36 with preservative; lyophilized.	**196-02-03**	**2 vials**
Positive Control – one vial of recombinant human sCD36; lyophilized.	**196-02-04**	**1 vial**
Streptavidin-HRP Conjugate – 220 µL/vial, 50-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB09**	**1 bottle**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB68C**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08C**	**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 1 month. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer, 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 (400 – 450 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 30 µL sample + 210 µL Dilution Buffer. A suggested 16-fold dilution is 15 µL sample + 225 µL Dilution Buffer.

Serum samples may require 16~64 fold dilution. A suggested 16-fold dilution is 20 µL sample + 300 µL Dilution Buffer. A suggested 32-fold dilution is 10 µL sample + 310 µL Dilution Buffer. A suggested 64-fold dilution is 5 µL sample + 315 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) - Dilution Buffer (DB09) is highly viscous, prior to use warm it in 30 - 37° C water bath until liquid flows more freely.

Soluble CD36 Standard - Reconstitute the Soluble CD36 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 128 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 #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **62.5 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	128 ng/ml
# 1	250µl of stock	250µl	62.5 ng/ml
# 2	250µl of 1	250µl	32 ng/ml
# 3	250µl of 2	250µl	16 ng/ml
# 4	250µl of 3	250µl	8 ng/ml
# 5	250µl of 4	250µl	4 ng/ml
# 6	250µl of 5	250µl	2 ng/ml
# 7	250µl of 6	250µl	1 ng/ml

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer.

Detection Antibody Concentrate – Reconstitute the Detection Antibody Concentrate with refer to lot of **Antibody Diluent Solution (DB68C)** to prepare a 10-fold concentrated solution. Pipette 5.4 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer the 0.6 mL of 10-fold concentrated solution to the tube to make 1x working solution. (two vials to prepare 12 ml of 1x working solution.)

Streptavidin-HRP Conjugate - Pipette 10.78 mL of **HRP Diluent Solution (DB08C)** into a 15 mL centrifuge tube and transfer 220 µL of 50-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 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 (400 – 450 rpm) 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 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker (400 – 450 rpm) 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 (400 – 450 rpm) 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 22-28 minutes on microplate shaker (400 – 450 rpm) 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 450nm within 3 minutes.

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 or 4-parameter curve fit.
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 62.5 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)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.096)
1	0.054
2	0.129
4	0.247
8	0.509
16	0.949
32	1.628
64	2.215

SPECIFICITY

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 dilutions, samples, or positive control to each well. Incubate 2 hours on the microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on microplate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 22-28 min on microplate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.