

Human CD 36 (Soluble) ELISA Kit

Cat. No.:DEIA6943

Pkg.Size:96 T

Intended use

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

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.

General Description

CD36 (Cluster of Differentiation 36) is an integral membrane protein found on the surface of many cell types in vertebrate animals and is also known as FAT, SCARB3, GP88, glycoprotein IV (gpIV) and glycoprotein IIIb (gpIIIb). CD36 is a member of the class B scavenger receptor family of cell surface proteins. CD36 binds many ligands including collagen, thrombospondin, erythrocytes parasitized with Plasmodium falciparum, oxidized low density lipoprotein, native lipoproteins, oxidized phospholipids, and long-chain fatty acids.

Principle Of The Test

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.

Reagents And Materials Provided

1. Human sCD36 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against sCD36: 1 plate
2. sCD36 Standard - 250 ng/vial of recombinant sCD36 in a buffered protein base with preservatives; lyophilized: 1 vial
3. Detection Antibody Concentrate - 105 ul/vial, 100-fold concentrated of biotinylated antibody against sCD36 with preservatives; lyophilized: 1 vial
4. Positive Control - one vial of recombinant human sCD36, lyophilized: 1 vial
5. Streptavidin-HRP Conjugate - 60 ul/vial, 200-fold concentrated solution with preservatives: 1 vial
6. Dilution Buffer - 30ml of buffered protein based solution with preservatives: 2 bottle
7. HRP Diluent Solution - 12 ml of buffered protein based solution with preservatives: 1 bottle
8. Wash Buffer - 50 ml of 10-fold concentrated buffered surfactant, with preservative: 1 bottle
9. TMB Substrate Solution -11 ml of TMB Substrate Solution: 1 bottle
10. Stop Solution - 11 ml of 0.5M HCl: 1 bottle

11. Plate Sealer: 1 piece
12. Plastic Pouch: 1 piece

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. Microplate shaker (250-300rpm).
3. Pipettes and pipette tips.
4. Deionized or distilled water.
5. Squirt bottle, manifold dispenser, or automated microplate washer.
6. 100 ml and 500 ml graduated cylinders.

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.

Specimen Collection And Handling

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at $1000 \times g$ within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. 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 $1000 \times g$. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. 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.

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. A suggested 64-fold dilution is 10 μl sample + 310 μl Dilution Buffer. Then, 150 μl 32-fold diluted sample + 150 μl Dilution Buffer.

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.

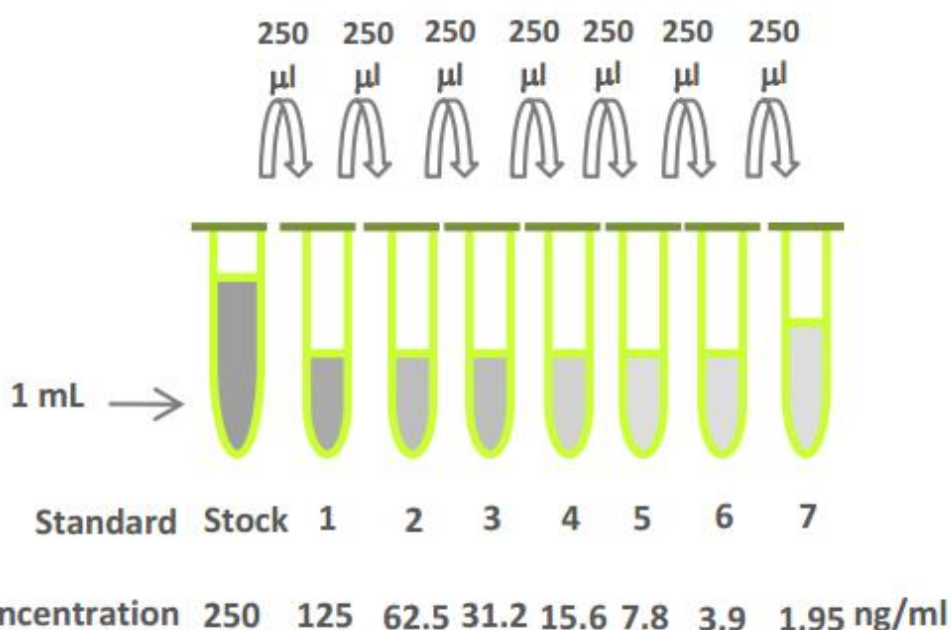
Dilution Buffer DB09 - If Dilution Buffer is highly viscous, warm in 27-30 °C water bath until liquid.

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 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 250 ng/ml standard serves as the high standard.

The Dilution Buffer serves as the zero standard (0 ng/ml).

Table 1.

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 L 100-fold Detection Antibody Concentrate to the tube to make working solution.

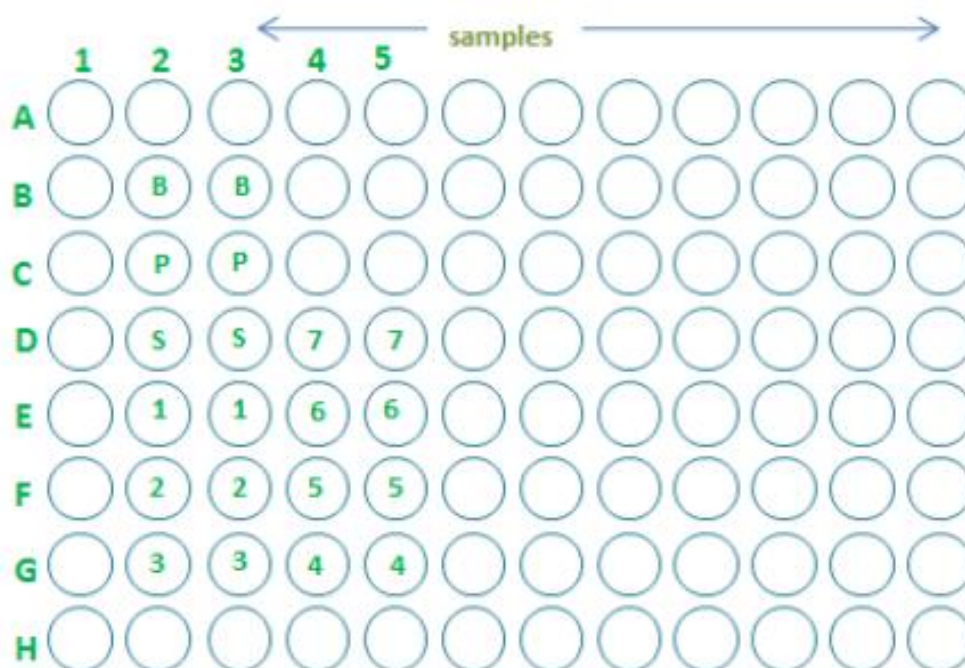
Streptavidin-HRP Conjugate - Pipette 11.94 ml of **HRP Diluent Solution (DB01)** into a 15 ml centrifuge tube and transfer 60 L of 200-fold concentrated stock solution to prepare working solution. **Note:** 1× working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 0.5 ml of Dilution Buffer. **Note:** Positive Control solution should be prepared and used within a few days.

Assay Steps

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 micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
 3. Add 100 ul of Dilution Buffer to Blank wells (F4, F5).
 4. Add 100 ul of Standard (from B2, B3 to G2, G3 and G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate 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 1× Wash Buffer (300 ul) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to 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 ul of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
 7. Repeat the aspiration/wash as in step 5.
 8. Add 100 ul of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. Protect from light.
 9. Repeat the aspiration/wash as in step 5.
 10. Add 100 ul of Substrate Solution to each well. Incubate for 2-5 minutes at room temperature. **Protect from light.**
 11. Add 100 ul 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 micro-plate reader set to 450 nm.
- Figure 1.



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 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 Standard Curve

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Table 2.

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

Positive Control: 3-11 ng/ml

Detection Range

1.95 - 250 ng/ml

Sensitivity

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of sCD36 was 1000 pg/ml.

Specificity

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

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 and assayed.
To assess the linearity of the assay, pooled human serum samples were diluted with Dilution Buffer and assayed.
Table 3.

pooled human EDTA plasma samples

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

pooled human serum samples

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

Reproducibility

Intra-assay Precision: 4-6%

Inter-assay Precision: 8-12%

Precautions

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.

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

Limitations

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.

Analyte Gene Information

Gene Name	CD36 CD36 molecule (thrombospondin receptor) [Homo sapiens]
Official Symbol	CD36
Synonyms	CD36; CD36 molecule (thrombospondin receptor); CD36 antigen (collagen type I receptor, thrombospondin receptor); platelet glycoprotein 4; FAT; GP3B; GP4; GPIV; SCARB3; GPIIB; PAS IV; PAS-4 protein; glycoprotein IIIb; cluster determinant 36; fatty acid translocase; platelet glycoprotein IV; scavenger receptor class B, member 3; leukocyte differentiation antigen CD36; CHDS7; PASIV; BDPLT10;
GeneID	948
mRNA Refseq	NM_000072
Protein Refseq	NP_000063
MIM	173510
UniProt ID	P16671
Chromosome Location	7q11.2
Pathway	Adaptive Immune System, organism-specific biosystem; Adipocytokine signaling pathway, organism-specific biosystem; Adipocytokine signaling pathway, conserved biosystem; Antigen processing-Cross presentation, organism-specific biosystem; Class I MHC mediated antigen processing & presentation, organism-specific biosystem; Cross-presentation of particulate exogenous antigens (phagosomes), organism-specific biosystem;
Function	Gram-negative bacterial cell surface binding; Gram-positive bacterial cell surface binding; high-density lipoprotein particle binding; lipid binding; lipoprotein particle binding; lipoteichoic acid receptor activity; low-density lipoprotein particle binding; low-density lipoprotein receptor activity; low-density lipoprotein receptor activity; receptor activity; thrombospondin receptor activity; transforming growth factor beta binding;

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