MOUSE SOLUBLE CD36 ELISA KIT

PURCHASE INFORMATION:

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

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

INTRODUCTION

Mouse sCD36 Immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Mouse SCD36 in cell culture supernates and plasma. It contains recombinant Mouse sCD36 and antibodies raised against this protein. It has been shown to accurately quantitie recombinant Mouse 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 Mouse sCD36.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal 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, an biotinylated polyclonal antibody specific for sCD36 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add 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 Calibrator Diluent 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 the appropriate Calibrator Diluent 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted sulfuric acid. Appropriate care, therefore, 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.

MATERIALS PROVIDED

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrated should be stored at -20 or -70 °C. Do not use past kit expiration date.
Opened / Reconstituted Reagents: *Reconstituted Standard (14 ng/ml) and Detection Antibody SHOULD BE STORED at -20 °C or – 70°C for up to one months.* Streptavidin - HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 6 months at 2 - 8° C.
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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.

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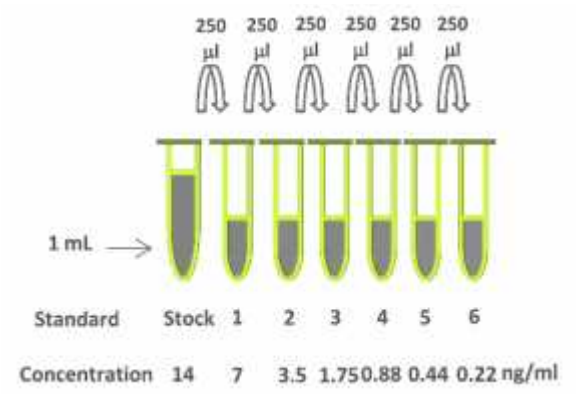
Serum: CD36 was expressed in plates. Activation of plates may increase sCD36 release. Serum samples cannot be used for sCD36 assay.

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.
sCD36 Standard - Refer to vial label for reconstitution volume. Reconstitute the **SCD36** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 14 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tube #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 14 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).



Detection Antibody- Reconstitute the **Detection Antibody concentrated** with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 11. 895 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer 105 µl of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11. 94 mL of Dilution Buffer into the 15 ml centrifuge tube and transfer 60 µl of 200-fold concentrated stock solution to prepare working solution. *Note: 1 x working solution of Streptavidin-HRP Conjugate should be used within a few days.*

Positive Control- Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. *Positive Control should be prepared and used immediately*.

ASSAY PROCEDURE

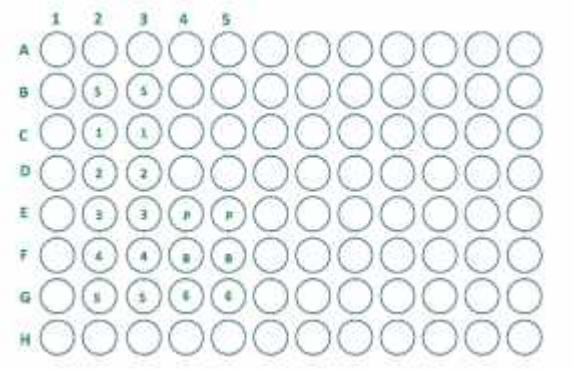
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

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 foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (F4, F5).
4. Add 100 µL of Standard (from B2 to G3, G4 to G5), sample, or control per well. Cover with the 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 Wash Buffer (300 µL) 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 µL 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 µL of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 40 minutes on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 20-25 minutes 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 micro-plate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, 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 sCD36concentrations 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.



TYPICAL DATA

These standard curves* are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Mouse CD36 Standard (pg/mL)	Average OD450 (Corrected)
213.75	0.038
437.5	0.071
875	0.128
1750	0.188
3500	0.358
7000	0.588
14000	0.821

*Lot No.: 20110193
** Positive Control (Lot No.: 2011093): 1892-3153 pg/ml

CALIBRATION

This immunoassay is calibrated against a highly purified CHO-expressed recombinant Mouse sCD36/Fc chimera.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of sCD36 Was 110 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant mouse CD36. The factors listed below were prepared at 200 ng/mL in Dilution Buffer, and assayed for cross reactivity

	Cross-reactivity
Mouse CD36/Fc chimera	100%
Human CD36/Fc chimera	15.6%
Human CD320	0
Rat sRAGE	0

REFERENCES

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3. Bell JA, et al. Lipid partitioning, incomplete fatty acid oxidation, and insulin signal transduction in primary human muscle cells: effects of severe obesity, fatty acid incubation, and fatty acid translocase/CD36 overexpression. J Clin Endocrinol Metab. 2010 Jul;95(7):3400-10. Epub 2010 Apr 28.
4. Sandoval JC, et al. Fenofibrate reduces postprandial hypertriglyceridemia in CD36 knockout mice. J Atheroscler Thromb. 2010 Jun 30;17(6):610-8. Epub 2010 Mar 30.
5. Abe T, et al. Key role of CD36 in Toll-like receptor 2 signaling in cerebral ischemia. Stroke. 2010 May;41(5):898-904. Epub 2010 Apr 1.
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SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards
Add 100µl 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 to each well. Incubate 2 hours on the plate shaker at RT.
Aspirate and wash 4 times.
Add 100 µl Streptatvin HRP conjugate to each well. Incubate 40 min on the plate shaker at RT.
Aspirate and wash 4 times.
Add 100 µl Substrate to each well. Incubate 20-25 min on the bench top. Protect from light.
Add 100 µl Stop Solution to each well. Read 450nm within 15 min