

MOUSE SOLUBLE CD36 ELISA KIT

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



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

PURCHASE INFORMATION:

ELISA NAME	MOUSE SCD36 ELISA
Catalog No.	SK00196-03
Lot No.	
Formulation	96 T
Standard range	0.22-14 ng/mL
Sensitivity	0.11 ng/mL
Sample Volume	100 µl
Dilution Factor	<i>Optimal dilutions should be determined by each laboratory for each application.</i>
Sample Type	EDTA Plasma, cell culture supernates
Specificity	Mouse sCD36
Intra-assay Precision	6-8%
Inter-assay Precision	10-12%
Storage	4 °C

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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 quantify 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, a 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 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

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_ 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 the appropriate 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric 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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Mouse CD36 Microplate - 96 well polystyrene microplate coated with a polyclonal antibody IgG against sCD36.	196-03-01	1 plate
SCD36 Standard – 14 ng/vial of recombinant Mouse sCD36 in a buffered protein base with preservatives; lyophilized.	196-03-02	1 vial
Detection Antibody Concentrate – 110 µL / vial, 100-fold concentrated of Biotinylated polyclonal IgG against SCD36 with preservatives; lyophilized.	196-03-03	1 vial
Positive Control - 1 vial of recombinant Mouse sCD36 in a buffered protein base with preservatives; lyophilized.	196-03-04	1 vial
Streptavidin-HRP Conjugate -60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Dilution Buffer - 60 mL of buffered protein based solution with preservatives	DB06	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 (0.5M HCl) , 11 ml of 0.5M HCl	S-STOP	1 bottle
Plate sealer.	EAPS	1 piece

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 kit past 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 month. Streptavidin - HRP Conjugate 200-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 pouch containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C.

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.

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

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.

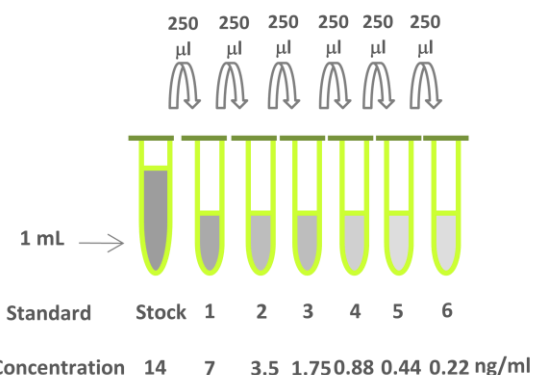
REAGENT PREPARATION

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).

TUBE	STANDARD	REAGENT DILUENT	CONCENTRATION
stock	powder	1 ml	14 ng/ml
# 1	250µl of stock	250µl	7 ng/ml
# 2	250µl of 1	250µl	3.5 ng/ml
# 3	250µl of 2	250µl	1.75 ng/ml
# 4	250µl of 3	250µl	0.875 ng/ml
# 5	250µl of 4	250µl	0.437 ng/ml
# 6	250µl of 5	250µl	0.218 ng/ml



Detection Antibody- Reconstitute the **Detection Antibody concentrated** with 110 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 11. 89 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and

TYPICAL DATA

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

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	0 (0.167)
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.:

** 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 0.11 ng/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

PROTEIN NAME	CROSS-REACTIVITY%
Mouse CD36/Fc chimera	100
Human CD36/Fc chimera	15.6
Human CD320	0
Rat sRAGE	0

REFERENCES

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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 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 min on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution 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