HUMAN SOLUBLE CD93 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE CD93 CONCENTRATIONS IN CELL CULTURE SUPERNATES, EDTA PLASMA AND SERUM

PURCHASE INFORMATION:

ELISA NAME	HUMAN SOLUBLE CD93 ELISA
Catalog No.	SK00399-01
Lot No.	
Formulation	96 T
Standard range	7.8 - 1000 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µL
Dilution Factor	800 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA plasma, Cell Culture Supernates
Specificity	Human soluble CD93
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C

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

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INTRODUCTION

Human soluble CD93 immunoassay is a solid phase ELISA designed to measure human sCD93 in cell culture supernates, serum, and plasma. It contains recombinant human sCD93 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human sCD93. Results obtained with naturally occurring sCD93 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 sCD93.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for sCD93 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sCD93 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for sCD93 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 sCD93 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 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
CD93 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against human sCD93.	**399-01-01**	**1 plate**
sCD93 Standard – 4000 pg/vial of recombinant human sCD93 in a buffered protein base with preservative; lyophilized.	**399-01-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of biotinylated purified antibody against human sCD93 with preservative; lyophilized.	**399-01-03**	**1 vial**
Positive Control – one vial of recombinant human sCD93; lyophilized.	**399-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB01**	**2 bottles**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**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 – 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 8 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 (stock) and Detection Antibody concentrated solution should be stored for up to two weeks at -70° C. Streptavidin-HRP Conjugate 100-fold concentrated solution **(protect from light)** and other components may be stored at 2 – 8° C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. 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.
- 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 or -70° 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 or -70° 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 or -70° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

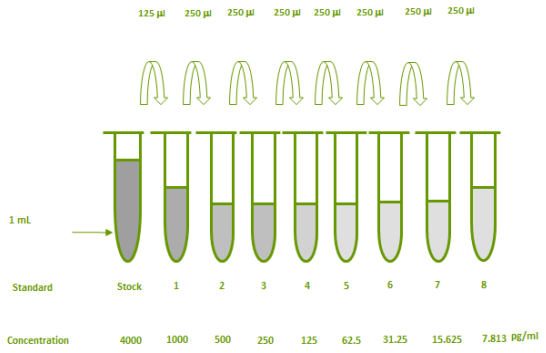
Serum and plasma samples may require an 800-fold dilution. A suggested 20-fold dilution is 5 µL sample + 95 µL Dilution Buffer. To make an 800-fold dilution, 6 µL of 20-fold diluted sample + 234 µL Dilution Buffer. **However, this is only a suggestion. A pretest is needed to determine the optimal dilutions of your samples 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 50mL of Wash Buffer Concentrate into deionized or distilled water (450mL) to prepare 500 mL of 1x Wash Buffer.

sCD93 Standard - Refer to vial label for reconstitution volume. Reconstitute the **sCD93 standard** with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 375 µL of Dilution Buffer into tube #1 and add 125 µL of 4000 pg/mL stock standard to make the high standard of 1000 pg/mL. Pipette 250 µl to tubes #2 to #8. Use the 1000 pg/mL standard solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
stock	powder	1.0 ml	4000 pg/ml
# 1	125µl of stock	375µl	1000 pg/ml
# 2	250µl of 1	250µl	500 pg/ml
# 3	250µl of 2	250µl	250 pg/ml
# 4	250µl of 3	250µl	125 pg/ml
# 5	250µl of 4	250µl	62.5 pg/ml
# 6	250µl of 5	250µl	31.25 pg/ml
# 7	250µl of 6	250µl	15.625 pg/ml
# 8	250µl of 7	250µl	7.813 pg/ml



Positive Control - Reconstitute **Positive Control** with 1.0 mL of Dilution Buffer. ***Note:*** *Positive Control*

could be reused in a couple of days if stored at -20° C and -70° C.

Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB06)** 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* ***(protect from light)****.*

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 duplicate.

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 with the desiccant pack.
3. Add 100 µL of **Dilution Buffer** to Blank wells (B2, B3).
4. Add 100 µL of **Standard solutions #8 - #1** in reverse order of serial dilution (D4, D5 to G4, G5 and G2, G3 to D2, D3), **sample,** or **positive control** (C2, C3) 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 **1x 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 plate 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 1 hour on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 5-10 minutes on microplate shaker 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 microplate reader set to 450 nm.

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 sCD93 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 1000 pg/mL may result in inaccurate, low human soluble CD93 levels. Such samples require further external predilution according to expected human soluble CD93 values with Dilution Buffer in order to precisely quantify the actual human soluble CD93 level.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human sCD93.

TYPICAL DATA

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

sCD93 Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.092)
7.813	0.018
15.625	0.031
31.25	0.056
62.5	0.116
125	0.229
250	0.410
500	0.697
1000	1.091

- **Lot No.:**
- **Positive Control:**
-

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human sCD93	100%
Human sCD36	0
Human sCD163	0
Human sCD209	0
Human sCD320	0
Human TNF-α	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to the 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 1 hour on plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 5-10 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.