

HUMAN SOLUBLE CD58 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN SOLUBLE CD58 CONCENTRATIONS IN
SERUM, PLASMA AND CELL CULTURES



ALWAYS REFER TO LOT SPECIFIC
PROTOCOL PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ AND CHECK ALL ITEMS OF EACH KIT
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 CD58 ELISA KIT
Catalog No.	SK00007-09B
Lot No.	20115239
Formulation	96 T
Standard range	125 - 8000 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma, Cell Cultures
Dilution Factor	4~8 (<i>Optimal dilutions should be determined by each laboratory for each application</i>)
Specificity	Human CD58
Calibration	human CD58 ECD (29-215) His Tag (HEK293 derived)
Intra-assay Precision	2 - 5%
Inter-assay Precision	4 - 8%
Storage	2 - 8° C for 8 months, see page 2 for more information
This kit contains sufficient materials to run approximately 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

Human Soluble CD58 is an endogenous glycoprotein ligand of the T-cell surface receptor CD2. The researchers reported human soluble CD58 is a novel circulating biomarker related to Acute Myeloid Leukemia (AML). Avisa Bioscience Manufactured soluble CD58 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural sCD58 from Serum and EDTA plasma samples in a sandwich ELISA format. The lysate of animal free HEK293 cells transfected with human CD58 cDNA can be detected by this ELISA Kit.

This immunoassay contains recombinant human CD58 derived from HEK293 animal free and high sensitivity antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CD58 in human samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human CD58. The capture antibody can bind to the CD58 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against CD58 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 is added to the wells and color develops in direct proportion to the amount of CD58 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

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

_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.

_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.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
CD58 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against CD58.	007-09-01	1 plate
CD58 Standard – 25.6 ng per vial of rh CD58 (HEK293 derived) in a buffered protein base with preservative; lyophilized.	007-09-02	1 vial
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrated of biotinylated antibody against CD58 with preservative; lyophilized.	007-09-03	1 vial
Positive Control - one vial of recombinant rh CD58; lyophilized.	007-09-04	1 vial
Streptavidin-HRP Conjugate – 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB90	1 bottle
Standard Reconstitute Solution – 1.5 mL of buffered protein based solution with preservative.	DB01	1 bottle
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB90B	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB08C	1 bottle
Wash Buffer 20X - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution.	TMB03	1 bottle
Stop Solution - 11 mL of 0.125M HCl solution.	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece

Plastic Pouch	P01	1 piece
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HUMAN SOLUBLE CD58 ELISA KIT SK00007-09B WAS PACKAGED BY 2 X 96T OF SK0007-09.

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 8 months. For longer storage up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and 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 (250 – 300 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

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.

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.

Optional: Use Aprotinin (enzyme inhibitor)
(Aviscera Bioscience’s Order Code: 00700-01-25, 25

TIU for 50 ml sample solution) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Human Serum or EDTA Plasma samples require 4 ~ 8 fold pre-dilution by Dilution Buffer. A suggested 4-fold dilution is 25 µl sample per well + 75 µl Dilution Buffer per well. A suggested final 8-fold dilution is 12.5 µl per well of sample solution + 87.5 µl per well of Dilution Buffer.

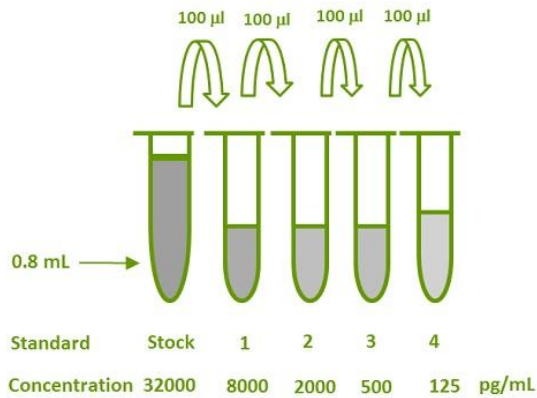
Optimal dilutions should be determined by each laboratory for each application with a sample pretest.
Use polypropylene test tubes.

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 **25 mL of Wash Buffer Concentrate 20X** into deionized or distilled water (**475 mL**) to prepare 500 mL of 1x Wash Buffer.

CD58 Standard - Reconstitute the Human CD58 standard with 0.8 mL of Standard Reconstitute Solution. This reconstitution produces a stock solution of 32000 pg/mL. Allow the standard to sit for a minimum of 3 minutes with gentle agitation prior to making dilutions. Pipette 300 µL of Dilution Buffer into the tube #1 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **8000 pg/mL** standard (or refer to specific lot) serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Store the stock solution of standard at -20 ~ - 70 °C for a few days.

TUBE		STANDARD RECONSTITUTE SOLUTION DB01	CONCENTRATION
stock	powder	0.8 ml	32000 pg/ml
	STANDARD	DILUTION BUFFER DB90	CONCENTRATION
# 1	100 µl of stock	300 µl	8000 pg/mL
# 2	100 µl of 1	300 µl	2000 pg/ml
# 3	100 µl of 2	300 µl	500 pg/ml
# 4	100 µl of 3	300 µl	125 pg/ml



Positive Control - Reconstitute the Positive Control with 2 mL of Dilution Buffer to prepare the working solution of positive control. Discard the working solution of positive control after use. It is for one time use only.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB90B)** to produce a 10-fold concentrated stock solution prior to add 1x detection antibody solution to each well (Step 5).

For 96 wells test, **freshly** pipette 9 mL of **Antibody Diluent Solution (DB90B)** into a 15 ml centrifuge tube and transfer 1 mL of 10-fold concentrated stock solution to prepare working solution. *If run a partial strip test, **freshly** prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 20-fold concentrated detection antibody at -20 °C for a few days. The 1x working solution should be used in 10 min.*

Streptavidin-HRP Conjugate - For 96 wells test **freshly** pipette 9.9 mL of **HRP Diluent solution (DB08C)** into a 15 mL centrifuge tube and transfer **100 µL of 100-fold concentrated** stock solution to prepare working solution prior to Step 7. **Protect from light.**

*The working solution of Streptavidin-HRP Conjugate **should be freshly prepared** and used within 10-15 min. If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock*

solution of 125-fold concentrated Streptavidin HRP ONLY at 2 -8°C for 12 months.

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 DB90 to to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, diluted samples, or positive control per well. Cover with plate sealer. Incubate for **2 hours** on microplate shaker at room temperature.
4. Discard the solution of each well by hold the plate and quickly invert the plate top down. Discard 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.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for **90 minutes** on microplate shaker at room temperature.
6. Repeat the discard/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for **45 minutes** on microplate shaker at room temperature. **Protect from light.**
8. Repeat the discard/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for **20~30 minutes** on microplate shaker 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 450 nm within 5 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve or 4-parameter fit to more accurately quantify the standard dilutions. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human soluble CD58 His Tag (HEK293)	100
Human CD2 (HEK293 derived)	0

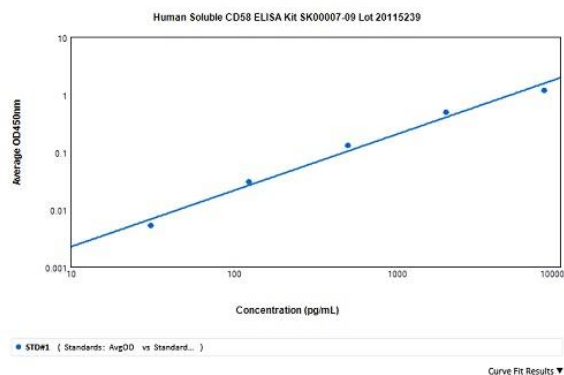
TYPICAL STANDARD CURVE

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

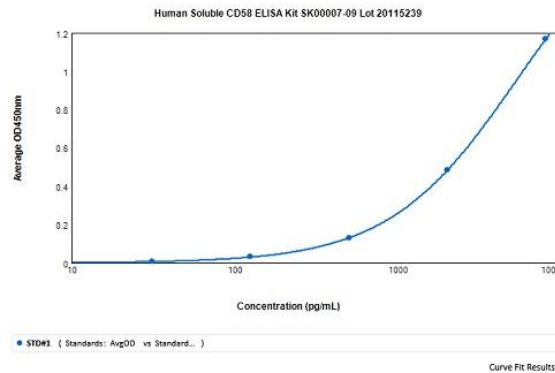
STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.087)
125	0.030
500	0.130
2000	0.483
8000	1.167

- Lot No.: 20115239
- Positive Control: 1000 ~ 4000 pg/mL

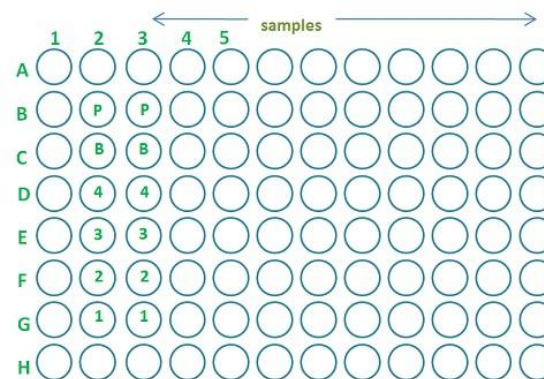
Standard Curve (125 ~ 8000 pg/mL) was fitted by Log-Log:



Standard Curve (125 ~ 8000 pg/mL) was fitted by 4-parameter:



Position of well



The research pooled human samples were pre-diluted with Dilution Buffer DB90. Its linearity and recovery was assayed By Human Soluble CD58ELISA Kit SK00007-09.

Sample Type	Dilution	Assayed (pg/mL)	Final (ng/mL)	Recovery %
Human Serum	4 X	2085.172	8340.688	100
Human Serum	8 X	1241.681	9933.446	119
Human EDTA Plasma	4 X	2253.004	9012.018	100
Human EDTA Plasma	8 X	1278.130	10225.04	114

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl per well of Dilution Buffer to blank wells. Add 100 µl of standard dilutions, diluted samples, or positive control to the well. Incubate for 2 hours on the plate shaker at RT.
↓
Discard and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate for 90 minutes on the plate shaker at RT.
↓
Discard and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate for 45 minutes on the plate shaker at RT. Protect from light.
↓
Discard and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate 20~30 minutes on the plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 5 minutes.

Human METRNL ELISA Kit	SK00478-06
Human Myonectin/CTRP15 ELISA Kit	SK00393-15
HS Soluble Neprilysin Human ELISA Kit	SK00724-01
Human Soluble LOX-1 ELISA Kit	SK00006-01
Human CHGA (19-131)/Vasostatin-2 ELISA Kit	SK00084-02

More info check

- <https://www.aviscerabioscience.net>

The reliable research Biomarker ELISA KITS were manufactured by Aviscera Bioscience for Research Use Only.

Biomarker Name	Catalog No.
Cholesin/C7ORF50 Human ELISA Kit	SK00027-09
Feimin/C5ORF24 Human ELISA Kit	SK00001-06
FAM19A1/TAFA1 Human ELISA Kit	SK00419-06
EPDR1 Human ELISA Kit	SK00023-06
LPGDS Human ELISA Kit	SK00025-06
Human Endotrophin ELISA Kit	SK00009-08
Nidogen-2 Human ELISA Kit	SK00480-06
Isthmin-1 Human ELISA Kit	SK00036-06
Human Irisin ELISA Kit	SK00170-08
HS BDNF (Human, Rat) ELISA Kit	SK00752-01
Human Sortilin ELISA Kit	SK00472-01
Human Lipocalin 13 ELISA Kit	SK00648-06