

SOLUBLE CD66C (HUMAN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN SOLUBLE CD66C CONCENTRATIONS IN
SERUM AND PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL
PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ 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	SOLUBLE CD66C (HUMAN) ELISA KIT
Catalog No.	SK00146-01
Lot No.	20114512
Formulation	96 T
Standard range	15.6 - 1000 pg/mL
Sensitivity	7 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma
Dilution Factor	<i>20-40 (Optimal dilutions should be determined by each laboratory for each application)</i>
Specificity	Human soluble CD66C
Calibration	Human CD66C recombinant, extracellular domain (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 - 8° C for 2 month, 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

This Human Soluble CD66C ELISA Kit contains the necessary components required for the quantitative measurement of natural and recombinant human CD66C from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CD66C derived from HEK293 cells animal free cultures and monoclonal antibody raised against this protein. Results from this immunoassay have shown to accurately quantify natural and recombinant human CD66C samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human soluble CD66C. The capture antibody can bind to the soluble CD66C in the standard and samples. After washing the plate of any unbound substances, a HRP conjugated monoclonal antibody against sCD66C is added to the wells. 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 sCD66C 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

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_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
sCD66C Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against soluble CD66C.	146-01-01	1 plate
Soluble CD66C Standard – 1000 pg/vial of recombinant soluble CD66C in a buffered protein base with preservative; lyophilized.	146-01-02	1 vial
Detection Antibody HRP Concentrate – 105 µL/vial, 100-fold concentrated of monoclonal antibody HRP conjugated against CD66C with preservative; lyophilized.	146-01-03	1 vial
Positive Control - one vial of recombinant CD66C; lyophilized.	146-01-04	1 vial
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB02	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB0208C	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 0.25M HCl solution.	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 2 months. For longer storage up to 10 months, unopened Standard, Positive Control, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Detection Antibody -HRP Conjugate Concentrate 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 (350 – 400 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). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples will require 20~ 40 fold dilution. A pretest is needed to optimize the dilution of samples. A 20 fold dilution is 15 μL of samples + 285 μL of Dilution Buffer. A 40 fold dilution is 50 μL per well of 20-fold diluted samples + 50 μL per well of Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera 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.

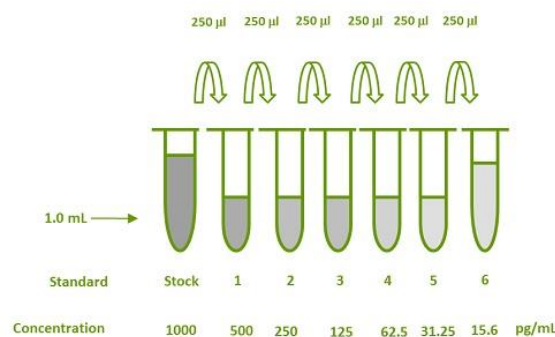
REAGENT PREPARATION

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 1x Wash Buffer.

Soluble CD66C Standard - Reconstitute the soluble CD66C standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 pg/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 the tube #1 to #6. Use the stock solution to produce a 2-fold 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). Store the stock solution at -70°C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	1000 pg/ml
# 1	250 μl of stock	250 μl	500 pg/ml
# 2	250 μl of 1	250 μl	250 pg/ml
# 3	250 μl of 2	250 μl	125 pg/ml
# 4	250 μl of 3	250 μl	62.5 pg/ml
# 5	250 μl of 4	250 μl	31.25 pg/ml
# 6	250 μl of 5	250 μl	15.6 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. Discard the positive control after use. It is for one time use only.

Detection Antibody HRP Conjugate- For 96 wells test, freshly pipette 10.395 mL of **Antibody Diluent Solution (DB08C)** into a 15 ml centrifuge tube and transfer 105 μL of 100-fold concentrated stock

solution to prepare working solution. For partial strip test, freshly prepare 900 μ L per well of the working solution. Always store the 100-fold concentrated stock solution at 2 ~ 8 °C for 10 months. The 1x working solution should be used in 20-30 min.

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 μ L per well of Dilution Buffer to Blank wells.
3. Add 100 μ L of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. 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 μ L) 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 μ L of Detection Antibody HRP conjugate working solution to each well. Cover with plate sealer. Incubate for 1 hour on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
9. Add 100 μ L of Substrate Solution to each well. Incubate for 20-25 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 μ L 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 2 min.

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 or 4-parameter curve 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

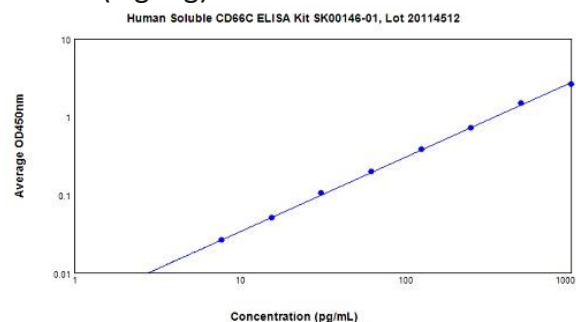
PROTEINS	CROSS-REACTIVITY (%)
Human Soluble CD66C (HEK293)	100
Human CD147 (HEK293 derived)	0
Human CD36 (HEK293)	0
Human sCD209	0
Human sCD305	0
Human sCD306	0

TYPICAL STANDARD CURVE

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

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.064)
7.8 (optional)	0.026
15.6	0.050
31.25	0.104
62.5	0.197
125	0.379
250	0.723
500	1.479
1000	2.639

- Lot No.: 20114512
- Positive Control: 60 ~ 250 pg/mL (log-log)



SUMMARY OF ASSAY PROCEDURE

Research Samples Assay

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody HRP working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate for 20- 25 min on the plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 2 min.

The research human serum or EDTA plasma samples were diluted by Dilution Buffer DB02. The linearity and recovery was assayed by Human Soluble CD66C ELISA Kit SK00146-01

Sample Type	Dilution Factors	Assayed (pg/ml)	Final Assay (ng/ml)	Recovery (%)
Serum	20 X	513.959	10.279	100
Serum	40 X	279.814	11.192	109
EDTA Plasma	20 X	174.308	3.486	100
EDTA Plasma	40 X	93.155	3.726	107

Well Position

