

HUMAN SOLUBLE CD19 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN SOLUBLE CD19
CONCENTRATIONS IN RECOMBINANT
PROTEIN, SERUM, EDTA PLASMA AND CELL
CULTURES.



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	HUMAN SOLUBLE CD19 ELISA KIT
Catalog No.	SK00431-01
Lot No.:	20115067
Formulation	96 T
Standard range	234 – 15000 pg/mL
Sensitivity	50 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Recombinant Protein, Serum, EDTA Plasma, Cell Cultures
Specificity	Human CD19 Extracellular Domain
Calibration	Human Soluble CD19 Rec. (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8°C for 8 months.
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 CD19 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CD19 from Recombinant Protein or Human cell cultures, Serum, EDTA Plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CD19 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural human CD19 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human CD19. The capture antibody can bind to the human CD19 in the standard and samples. After washing the plate of any unbound substances, another monoclonal antibody-HRP conjugate against human CD19 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 human CD19 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.

_Each laboratory must determine the optimal dilution factors for 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
CD19 Microplate – 96 well microplate coated with a monoclonal antibody specific for human CD19.	431-01-01	1 plate
CD19 Standard – 15 ng/vial of lyophilized recombinant human DLL4.	431-01-02	1 vial
Detection Antibody-HRP Conjugate – 105 µL/vial of 100-fold concentrated solution of monoclonal antibody conjugated to HRP against human CD19.	431-01-03	1 vial
Positive Control – one vial of lyophilized recombinant human CD19.	431-01-04	1 vial
Dilution Buffer - 45 mL of buffered solution with preservative.	DB01	1 bottle
Antibody Diluent Solution - 12 mL of buffered solution with preservative.	DB02	1 bottle
Wash Buffer 20X - 25mL 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 0.25M 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 up to 12 months, unopened Standard, Positive Control and Dilution Buffer (DB01) should be stored at -20°C or -70°C. **Detection Antibody-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 (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

CD19 Recombinant derived from HEK293 animal free.

HEK293 Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. 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 $\leq -20^{\circ}\text{C}$. 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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Other sample types, such as serum and plasma, need to be validated prior to use.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

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

CD19 Standard - Reconstitute the CD19 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 15 ng/mL. Allow the stock standard to sit for at least 5 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **15 ng/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 ml	15 ng/ml
# 1	100 μl of stock	300 μl	3.75 ng/ml
# 2	100 μl of 1	300 μl	0.938 ng/ml
# 3	100 μl of 2	300 μl	0.234 pg/ml

Positive Control - Reconstitute the Positive Control with 0.5 mL of Dilution Buffer.

Detection Antibody-HRP Conjugate – Freshly Pipette **9.9 mL** of **Antibody Diluent Solution** into a 15 mL centrifuge tube and transfer **100 μL of 100-fold** concentrated stock solution to prepare working solution (**protect from light**). **DO NOT FREEZE**. The 1 x working solution should be used in 10 ~ 15 min.

ELISA PROTOCOL

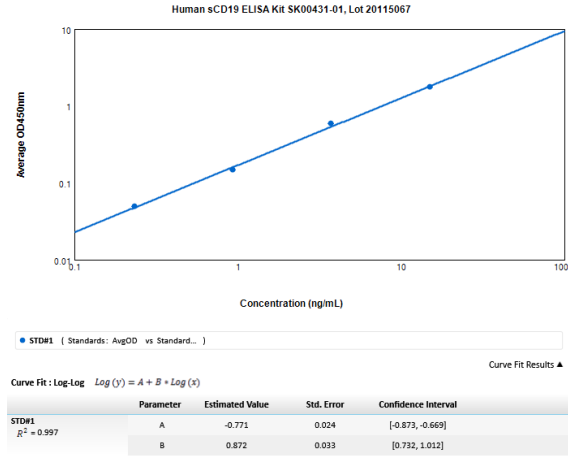
Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 μl per well of Dilution Buffer to Blank wells.
3. Add 100 μl per well of standard dilutions from #3 to #5 (reverse order of serial dilution), positive control or samples. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
4. Aspirate wells and wash 4 times with 300 μl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.

5. Add 100 µl per well of 1x Detection Antibody- HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 90 minutes on microplate shaker (250 rpm). **Protect from light.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Substrate Solution to each well. Incubate for 25-30 minutes on microplate shaker at room temperature. **Protect from light.**
8. 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.
9. Determine the optical density of each well using a microplate reader set to 450 nm within 5 min.

- Lot No.: 20115067
- Positive control (0.5 mL): 1.2~ 5 ng/mL (log-log)

Standard curve fit by log-log (0.234 ~ 15 ng/ml)

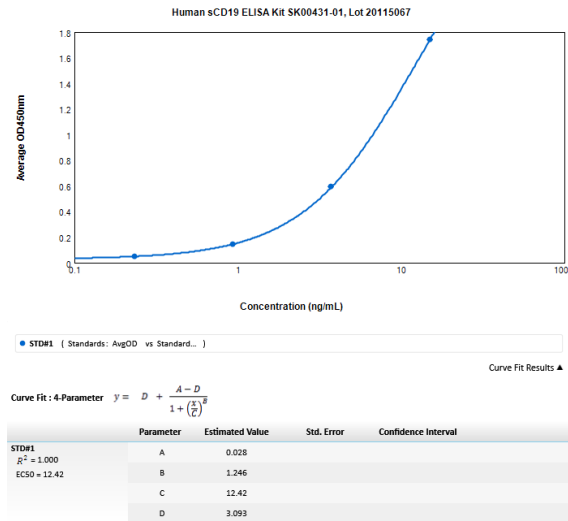


CALCULATION OF RESULTS

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.

Standard curve fit by 4-parameter (0.234 ~15 ng/ml):



SPECIFICITY

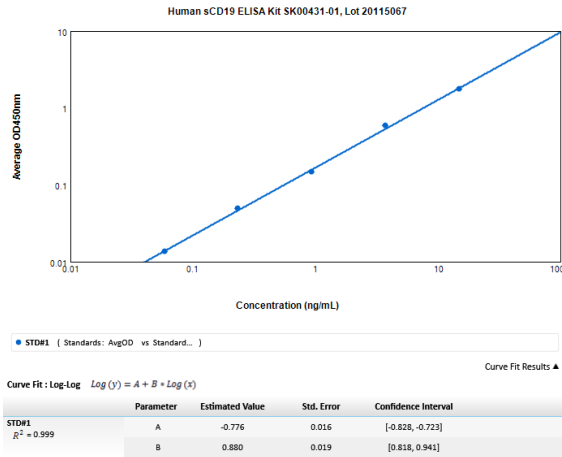
PROTEINS	CROSS-REACTIVITY
Human Soluble CD19 (HEK293)	100%
Human CD209	0
Human CD320	0

TYPICAL DATA

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

STANDARD (NG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.097)
0.059 (optional)	0.014
0.234	0.049
0.938	0.146
3.75	0.591
15	1.740

Standard curve fit by log-log (0.059 ~ 15 ng/ml)
(Optional)



Reliable Precision Biomarker ELISA Kits
were manufactured by Aviscera Bioscience.

HS CSF1R (H) ELISA Kit	SK00144-06
HS Endotrophin (H) ELISA Kit	SK00009-08
DLL4 (H) ELISA Kit	SK00538-01
CD146 (H) ELISA Kit	SK00628-01
HS BDNF (H,R) ELISA Kit	SK00752-01
HS ProBDNF (H) ELISA Kit	SK00752-09

SUMMARY OF ASSAY PROCEDURE

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 per well 1x Detection Antibody-HRP working solution to each well. Incubate 90 minutes on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl TMB Substrate Solution to each well. Incubate 25-30 min on the plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 5 min.

Research Sample Determination

The pooled research human serum samples were assayed by Human sCD19 ELISA Kit SK00431-01 at 0.725 ng/mL.

The pooled research human EDTA samples were assayed by Human sCD19 ELISA Kit SK00431-01 at 0.839 ng/mL.