HUMAN SOLUBLE CD21 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE CD21 CONCENTRATIONS IN SERUM AND PLASMA

PRODUCT INFORMATION:

ELISA NAME	HUMAN SOLUBLE CD21 ELISA KIT
Catalog No.	SK00620-01
Lot No.:	
Formulation	96 T
Standard range	31 - 2000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µL of diluted samples
Dilution Factor	200 ~ 400 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma
Specificity	Human CD21
Calibration	Human CD21 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C

This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

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DESCRIPTION

This Human CD21 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CD21 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CD21 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CD21 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human soluble CD21. The capture antibody can bind to the human soluble CD21 in the standard and samples. After washing the plate of any unbound substances, an antibody-HRP conjugate against human soluble CD21 is added to the wells. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human soluble CD21 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 with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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
sCD21 Microplate – 96 well microplate precoated with an anti-human soluble CD21 antibody.	**620-01-01**	**1 plate**
sCD21 Standard – 2000 pg/vial of recombinant human soluble CD21 in a buffered protein base with preservative; lyophilized.	**620-01-02**	**1 vial**
Detection Antibody-HRP Conjugate – 55 μL/vial of 200-fold concentrated solution of antibody conjugated to HRP against soluble CD21.	**620-01-03**	**1 vial**
Positive Control – one vial of recombinant human soluble CD21; lyophilized (optional).	**620-01-04**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	**DB10**	**2 bottles**
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-HRP Conjugate 200-fold concentrated solution should be stored at -20° C or -70° C. Substrate Solution can be stored at 2 – 8° C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components can be stored at 2 – 8° C for up to 8 months. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution SHOULD BE STORED at -20° C or -70° C for up to one month.

Microplate Wells: Return unused microplate strips to the plastic pouch with the desiccant pack.

Microplate may be stored for up to 6 months at 2 – 8 °C after opening.

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

SAMPLE PREPARATION

Serum and EDTA plasma samples may require a 200 ~ 400-fold dilution.

A suggested 10 -fold dilution is 10 μL of sample + 90 μL of Dilution Buffer. Then, to make a 200-fold dilution is 12 μL of 10-fold diluted samples + 228 μl of Dilution Buffer. To make a 400-fold dilution is 6 μL of 10-fold diluted sample + 234 μL of Dilution Buffer.

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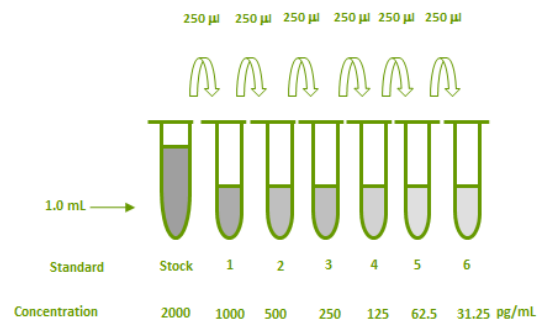
REAGENT PREPARATION

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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

sCD21 Standard - Reconstitute the sCD21 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **2000 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	2000 pg/ml
# 1	250μl of stock	250μl	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

| | 250 μl | 250 μl | 250 μl | 250 μl | 250 μl | 250 μl |

1.0 mL →						
Standard	Stock	1	2	3	4	5	6
Concentration	2000	1000	500	250	125	62.5	31.25 pg/mL

Positive Control - Reconstitute the Positive Control with 0.5 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20 °C or -70 °C.

Detection Antibody-HRP Conjugate - Pipette 10.945 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 55 μL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Detection Antibody-HRP

conjugate should be used within a few days **(protect from light)**. **DO NOT FREEZE.**

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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 μl per well of Dilution Buffer to Blank wells.
4. Add 100 μl per well of standard dilutions from #6 to #S (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).
5. Aspirate wells and wash 4 times with 300 μl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 μl per well of 1x Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 1 hour on microplate shaker (250 rpm). **Protect from light.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μL of Substrate Solution to each well. Incubate for 8-15 minutes on microplate shaker at room temperature. **Protect from light**.
9. 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

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 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.

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 (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.081)
31.25	0.018
62.5	0.052
125	0.086
250	0.182
500	0.330
1000	0.625
2000	1.339

- Lot No.:
- Positive control:

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human soluble CD21	100
Human CD44	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 per well 1x Detection Antibody-HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate 8-15 min on the plate shaker at RT. **Protect from light.**
⬇
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.