



Human Complement C5a ELISA Kit

Catalog Number: CEA-B261

Assay Tests: 96 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing your experiment.

Product information

This kit is specifically designed for the accurate quantitation of human Complement C5a from cell culture supernates, serum and plasma. **It has no cross-reactivity with native human Complement C5 protein.**

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

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Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA261-C01	Pre-coated Anti-Complement C5a Antibody Microplate	1 plate
CEA261-C02	Human Complement C5a Standard	20 µg×2
CEA261-C03	Biotin-Anti-Complement C5a Antibody Con. Solution	300 µL
CEA261-C04	Biotin-Antibody Dilution Buffer	8 mL
CEA261-C05	Streptavidin-HRP Con. Solution	500 µL
CEA261-C06	Streptavidin-HRP Dilution Buffer	15 mL
CEA261-C07	20× Washing Buffer	50 mL
CEA261-C08	Sample Dilution Buffer	15 mL×2
CEA261-C09	Substrate Solution	12 mL
CEA261-C10	Stop Solution	6 mL

NOTE: Bubbles in microplate wells do not affect the experiment and require no action. Proceed with the experimental procedures and methods described below.

Storage

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date.

For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

Contents	Storage conditions
Pre-coated Anti-Complement C5a Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C.
Human Complement C5a Standard	Aliquot and store for up to 1 month at -70°C in a freezer. Limit to one freeze-thaw cycle.

NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

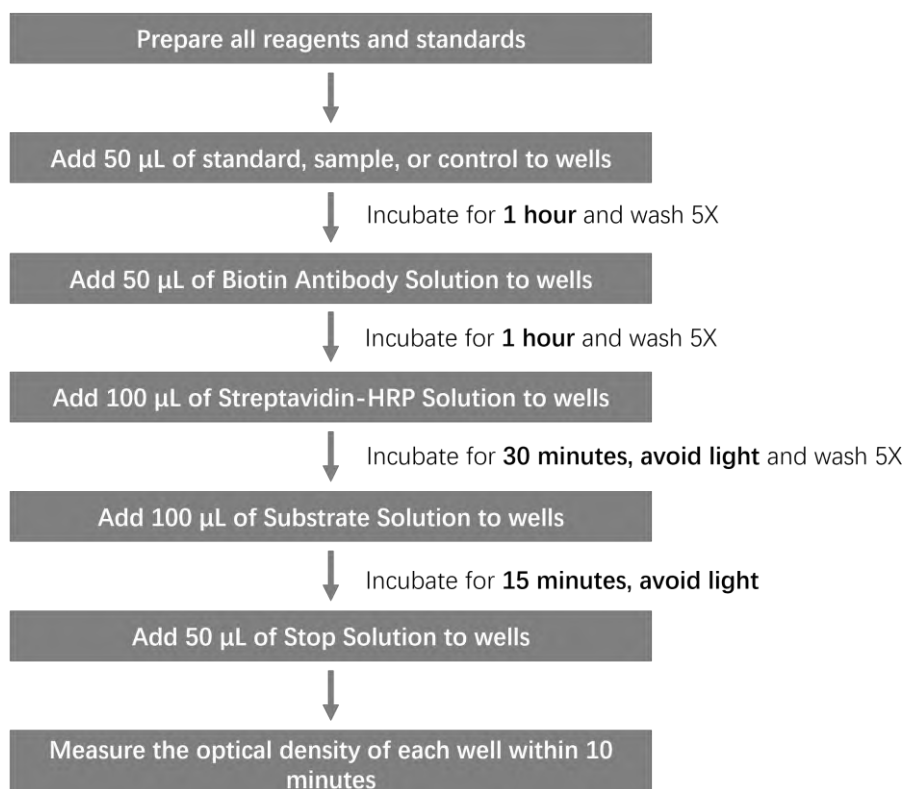
IMPORTANT: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in a 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

Required materials not supplied.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure or distilled water
Consumables	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

Workflow

Analyte: Complement C5a



NOTE: Incubation temperature is 18 °C-25 °C

Prepare the working buffers and standard dilutions.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin Antibody Solution: Add 240 µL of Biotin-Anti-Complement C5a Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. Streptavidin-HRP Solution: Add 400 µL of Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

Prepare the reconstituted standard.

Add 1 mL ultrapure water to the provided lyophilized product (CEA261-C02), dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted human Complement C5a Standard is 20 µg/mL.

NOTE: Avoiding vigorous shaking or vortexing. **Limit to one freeze-thaw cycle.**

Prepare the standard serial dilutions.

1. Label a tube "Cm". Add 5 µL of the reconstituted human Complement C5a Standard and 495 µL of Sample Dilution Buffer to tube Cm, gently mix well.
2. Label 7 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
3. Add 25 µL of the liquid from **Cm** and 975 µL of Sample Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 = 5000 pg/mL).
4. Prepare serial dilutions for the standard curve as follows: Add 500 µL of Sample Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).
5. Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 2500 pg/mL).
6. Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7.
7. Sample Dilution Buffer serves as zero standard (blank).

Prepare the sample.

Label a tube "S-1", add 20 µL of the serum specimen and 40 µL of Sample Dilution Buffer to tube S-1, mix gently well. The liquid from S-1 used to Complement C5a detection.

PROCEDURE OF ASSAY

1. Add 50 µL of Complement C5a Standard, sample, or control to wells. Incubate at room temperature (18-25 °C) for **1 hour**.
2. Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
3. Add 50 µL Biotin Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hour**.
4. Repeat step 2.
5. Add 100 µL of Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **30 minutes, avoid light**.
6. Repeat step 2.
7. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
8. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.
9. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes.

Note: the color in the wells should change from blue to yellow.

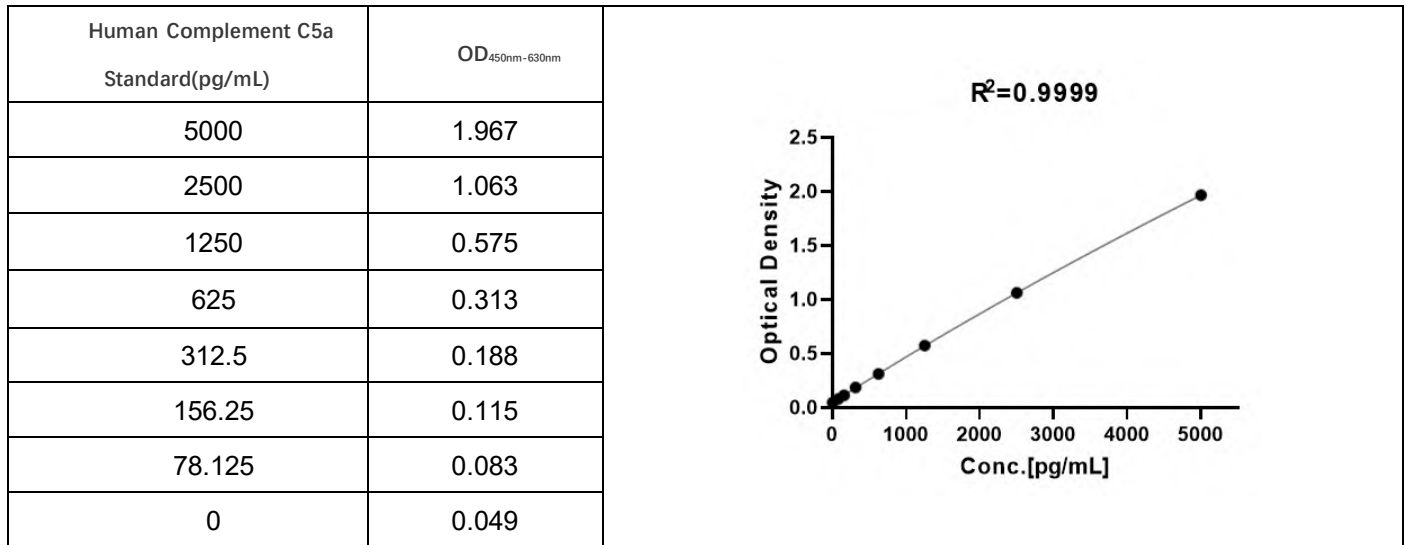
Note: To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.

CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample. Then, subtract the average optical density (O.D.) of the zero standard(blank).
2. Establish a standard curve by processing the data using computer software capable of executing a **four-parameter logistic (4-PL)** curve fitting.
3. Normal range of Standard curve: $R^2 \geq 0.9900$.
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

Typical data

Note: For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.



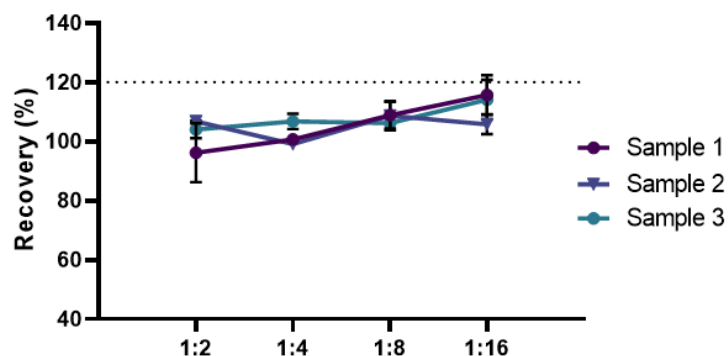
PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of Complement C5a is typically less than 60 pg/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

2. Linearity

Three samples (Serum) spiked with high concentrations of Complement C5a were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of Complement C5a for serum samples is 106.1%.



3. Intra-Assay Precision

Ten replicates of each of 3 samples containing different Complement C5a concentrations were tested in one assay. Acceptable criteria: CV < 10%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
2500	2239.5	115.3	10	5.1
1250	1122.1	81.2	10	7.2
625	533.9	28.0	10	5.2

4. Inter-Assay Precision

3 samples containing different concentrations of Complement C5a were tested in independent assays. Acceptable criteria: CV<15%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
2500	2643.1	195.1	9	7.4
1250	1204.9	114.5	9	9.5
625	652.6	56.7	9	8.7

5. Recovery

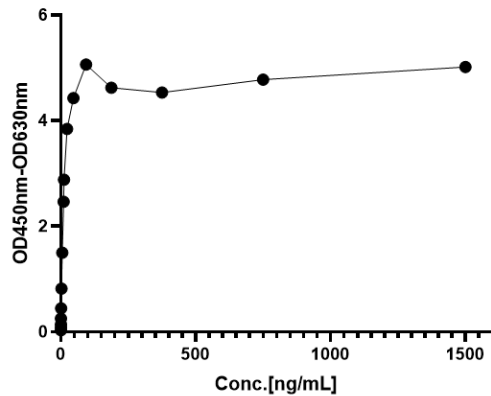
Recombinant Complement C5a was spiked into 3 human serum samples, and then analyzed. The average recovery of Complement C5a for serum samples is 92.5%.

Sample ID	Conc Measured (pg/mL)	Conc Added (pg/mL)	Conc Recovered (pg/mL)	Recovery (%)
1	2849.9	2500	2425.1	97.0
	1568.1	1250	1143.3	91.5
	989.5	625	564.7	90.3
	472.0	--	--	--
2	2933.8	2500	2426.0	97.0
	1639.0	1250	1131.2	90.5
	1128.5	625	620.7	99.3
	564.2	--	--	--
	3951.7	2500	2409.8	96.4

3	2651.9	1250	1110.0	88.8
	2050.2	625	508.3	81.3
	1713.2	--	--	--

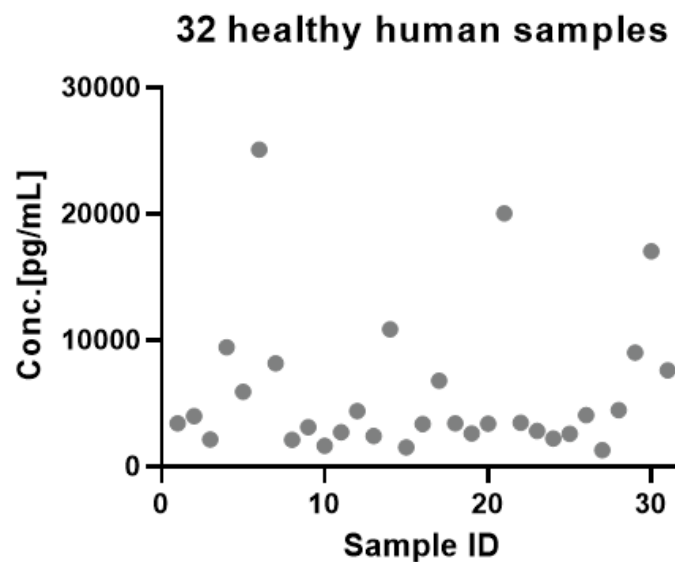
6. Hook Effect

Not be affected by the concentration of Complement C5a up to 46 ng/ml.



7. Sample Values

Healthy donor serum samples (n=32) were evaluated for the concentrations of human Complement C5a in the assay. The detectability rate of the samples was 100%, with a mean detectable concentration of 5.776 ng/mL and a concentration range of 1.299 to 25.084 ng/mL.



TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts