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ab193695 Complement C5a Human ELISA Kit

For the quantitative measurement of Human C5a in serum, plasma and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's Complement C5a Human ELISA Kit (ab193695) is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human C5A in serum, plasma and cell culture supernatants.

This assay employs an antibody specific for Human C5a coated on a 96-well plate. Standards and samples are pipetted into the wells and the immobilized antibody captures C5a present in the samples. The wells are washed and biotinylated anti-Human C5a antibody is added. After washing away any unbound biotinylated antibody, an HRP-conjugated streptavidin is pipetted to the wells. After incubation, the wells are again washed, followed by the addition of a TMB substrate solution to the wells. Color will develop in proportion to the amount of C5a bound in each well. Addition of the Stop Solution will change the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standard or sample to each well used. Incubate at room temperature.

Add prepared biotin antibody to each well. Incubate at room temperature.

Add prepared Streptavidin solution. Incubate at room temperature.

Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read at 450nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Pre-coated C5a microplate (12 x 8 well strips)	96 wells	-20°C
20X Wash Buffer Concentrate	25 mL	-20°C
Human C5a Standard	2 vials	-20°C
Assay Diluent A	30 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
Detection Antibody C5a (biotinylated anti-Human C5a)	2 vials	-20°C
200X HRP-Streptavidin concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Completely aspirate all solutions and buffers during wash steps.
- When preparing your standards, it is critical to briefly centrifuge the vial first. The powder may adhere to the cap and not be included in the standard solution resulting in an incorrect concentration. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and centrifuge briefly; repeat this procedure 3-4 times. This is an effective technique for thorough mixing of the standard without using excessive mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Assay Diluent B

5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

9.2 1X Wash Solution

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into 380 mL deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.3 Detection Antibody C5a (biotinylated anti-Human C5a)

Briefly centrifuge the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly centrifuge the 200X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. The 200X HRP-Streptavidin concentrate should be diluted 200-fold with 1X Assay Diluent B.

For example: Briefly centrifuge the vial and pipette up and down to mix gently. Add 50 µL of HRP-Streptavidin concentrate into a tube with 10 mL 1X Assay Diluent B to prepare a 1X HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix well.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.

10.1 Briefly centrifuge the vial of Human C5a Standard and then add 400 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture supernatants) into the Human C5a Standard vial to prepare a 50 ng/mL **Stock Standard**. Mix thoroughly but gently.

10.2 Label tubes #1-8.

10.3 Prepare the 2000 pg/mL **Standard #1** by adding 40 µL Stock Standard into tube #1 along with 960 µL Assay Diluent A or 1x Assay Diluent B. Mix thoroughly but gently.

10.4 Add 300 µL Assay Diluent A or 1x Assay Diluent B into tubes 2-8.

10.5 Prepare **Standard #2** by adding 300 µL Standard #1 to tube #2. Mix thoroughly but gently.

10.6 Prepare **Standard #3** by adding 300 µL from Standard #2 to tube #3. Mix thoroughly but gently.

10.7 Using the table below as a guide, prepare further serial dilutions.

10.8 Standard #8 contains no protein and is the Blank control.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	-	40	50,000	2,000
2	300 µL Standard #1	300	2,000	1,000
3	300 µL Standard #2	300	1,000	500
4	300 µL Standard #3	300	500	250
5	300 µL Standard #4	300	250	125
6	300 µL Standard #5	300	125	62.50
7	300 µL Standard #6	300	62.50	31.25
8	-	300	0	0

11. Sample Preparation

General Sample Information:

- If your samples need to be diluted, Assay Diluent A should be used for dilution of serum/plasma samples. 1X Assay Diluent B should be used for dilution of culture supernatants.
- Suggested dilution for normal serum/plasma: 40-400-fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Add 100 µL of each standard (see Standard Preparations, section) and sample into appropriate wells. Cover plate and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

13.2 Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with 300 µL 1X Wash Buffer using a multi-channel pipette or automatic plate washer. 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 by tapping gently against clean paper towels.

13.3 Add 100 µL of the prepared biotinylated Human C5a Detection Antibody (see Reagent Preparation section) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of prepared 1X HRP-Streptavidin solution (see Reagent Preparation section) to each well. Incubate for 45 minutes at room temperature with gentle shaking

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

13.8 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

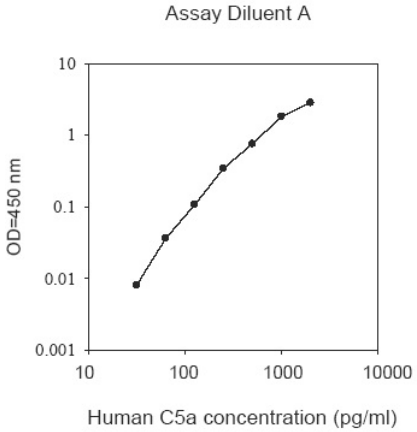

Figure 1. Example of typical human C5a standard curve using Assay Diluent A. The standard curve was prepared as described in Section 10.

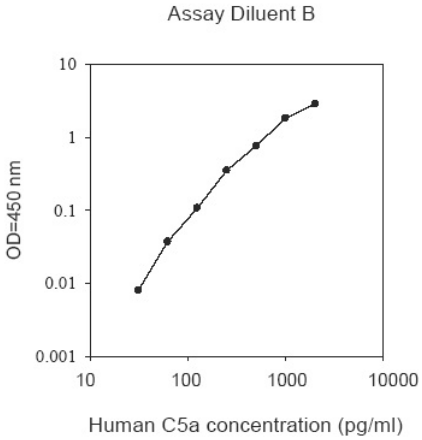

Figure 2. Example of typical human C5a standard curve using 1X Assay Diluent B. The standard curve was prepared as described in Section 10.

16. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of C5a is 31 pg/mL.

RECOVERY –

Recovery was determined by spiking various levels of Human C5a into Human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	127.1	122-138
Plasma	122.5	114-130
Cell culture media	92.43	70-104

LINEARITY OF DILUTION –

Serum Dilution	Average % Expected Value	Range (%)
1:2	105.0	97-113
1:4	98.36	93-104

Plasma Dilution	Average % Expected Value	Range (%)
1:2	100.4	92-108
1:4	92.01	84-100

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	104.7	97-113
1:4	103.1	95-111

PRECISION –

	Intra-Assay	Inter-Assay
CV (%)	<10%	<12%

17. Assay Specificity

The antibodies used within this ELISA kit detect Human C5a.

The antibodies do not cross-react with rhC3a, rha2-macroglobulin, rmC5a, or rmC5d.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829