ab279352 – Human C3a ELISA Kit

For the quantitative measurement of C3a in Human Serum, Plasma and Cell culture supernatants.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab279352

Storage and Stability:

Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. Observe the storage conditions for individual prepared components in the Reagent Preparation

Materials Supplied

Item	Quantity	Storage Condition
C3a Microplate	1 unit	-20°C
20X Wash Buffer	25 mL	-20°C
Human C3a Standard (Lyophilised)	2 vials	-20°C
Biotinylated anti-Human C3a detection antibody	2 vials	-20°C
200X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB Substrate Solution	12 mL	-20°C
Stop Solution	8 mL	-20°C
5X Assay Diluent	15 mL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize 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 litre graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare standard or sample dilutions

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.

5X Assay Diluent: Dilute 5X Assay Diluent 5-fold with deionized or distilled water before use. 1X Assay Diluent should be used to dilute serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 40,000 fold
Note: Levels of C3a may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
Biotinylated anti-Human C3a detection antibody: Briefly spin the vial before use. Add 100 µL of 1X Assay Diluent 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.

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

HRP-Streptavidin Concentrate: Briefly spin the vial of HRP-Streptavidin concentrate before use. HRP-Streptavidin should be diluted 200-fold with 1X Assay Diluent. For example: Briefly spin 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 to prepare a 200-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Standard Preparation

Always prepare a fresh set of standards for every use.
Discard working standard dilutions after use as they do not store well.
The following section describes the preparation of a standard curve for duplicate measurements (recommended).

- Briefly spin the Standard Vial.
- Add 400 µL of 1X Assay Diluent into Standard Vial to prepare a 50 ng/mL Standard stock solution. Gently mix the powder to allow it to dissolve thoroughly.
- Add 40 µL of Standard stock solution into a tube with 460 µl 1X Assay Diluent to prepare a 4000 pg/ml standard solution.
- Pipette 250 µL 1X Assay Diluent into each tube.
- Use the 4000 pg/mL Standard solution to produce a dilution series. Adding 250 µL from #1 to #2, then from #2 to #3, etc
- Mix each tube thoroughly before the next transfer.
- Tube #8 contains no protein and is the Blank control

Tube #	Volume to dilute	Volume of 1X Assay Diluent	Final Concentration pg/mL
1	4000 pg/mL standard stock solution	---	4000
2	250 µL of tube #1	250 µL	2000
3	250 µL of tube #2	250 µL	1000
4	250 µL of tube #3	250 µL	500
5	250 µL of tube #4	250 µL	250
6	250 µL of tube #5	250 µL	125
7	250 µL of tube #6	250 µL	62.5
8	--	250 µL	0

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. Prepare all reagents, working standards, and samples as directed in the previous sections. **Note:** 1X Assay Diluent should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 40,000 fold.

1. Label removable 8-well strips as appropriate for your experiment.

2. Add 100 µL of standard or sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature with gentle shaking.

3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multichannel Pipette or auto-washer. Complete removal of liquid at each step is essential for 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.

4. Add 100 µL of 1X prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 µL of prepared Streptavidin solution each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 3.

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

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

Calculation of results

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 or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

For all technical or commercial enquiries please go to:
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Version 1c | 21 April 2026