ab157711 Complement C3 Mouse ELISA Kit

For the quantitative measurement of mouse complement C3 in plasma and serum. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab157711
(use abcam.cn/ab157711 for China, or abcam.co.jp/ab157711 for Japan)

Materials Supplied and Storage

Store kit at +4°C immediately upon receipt. 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.

Item	Quantity	Storage Condition
Mouse Complement C3 ELISA Microplate	96 wells	4°C
Mouse Complement C3 Calibrator (lyophilized)	1 vial	4°C
5X Diluent Concentrate	50 mL	4°C
20X Wash Buffer Concentrate	50 mL	4°C
100X Enzyme-Antibody Conjugate	150 µL	4°C
Chromogen Substrate Solution	12 mL	4°C
Stop Solution	12 mL	4°C

Materials Required, Not Supplied

- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

1. 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 experiment.

1.1 1X Diluent Solution: The diluent solution is supplied as 5X Diluent Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O). The 1X Diluent Solution is stable for at least one week from the date of preparation and should be stored at 4°C.

1.2 1X Wash Buffer: The wash solution is supplied as 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30 - 35°C before dilution can dissolve crystals. The 1X Wash Buffer is stable for at least one week from the date of preparation and can be stored at room temperature (16 - 25°C) or at 4°C.

1.3 1X Enzyme-Antibody Conjugate: Calculate the required amount of 1X Enzyme-Antibody Conjugate solution for each microtitre plate test strip by adding 10 µL 100X Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming. The working conjugate solution is stable for up to 1 hour when stored in the dark.

1.4 Chromogen Substrate Solution: Ready to use as supplied.

1.5 Stop Solution: Ready to use as supplied.

2. Standard Preparation

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use

2.1 Add 1.0 mL of distilled or de-ionized water to the Mouse Complement C3 Calibrator and mix gently until dissolved. The calibrator is now at the concentration stated on the vial. **ΔNote**: The reconstituted Mouse Complement C3 Calibrator should be aliquoted and stored frozen. Avoid multiple freeze-thaw cycles.

2.2 Label tube numbers 1 – 7.

2.3 Prepare **Standard #1** by adding the appropriate volume of 1X Diluent Solution (see below) to tube #1. Add 60 µL of stock Mouse Complement C3 Calibrator to obtain a concentration at 200 ng/mL and mix thoroughly and gently.

***Example:**
NOTE: This example is for demonstration purposes only. Please remember to check your calibrator vial for the actual concentration of calibrator provided.
C_S = Starting concentration of reconstituted Mouse Complement C3 Calibrator (variable e.g. 2.74 µg/mL)
C_F = Final concentration of Mouse Complement C3 Calibrator for the assay procedure (200 ng/mL)
V_A = Total volume of stock Mouse Complement C3 Calibrator to dilute (e.g. 60 µL)
V_D = Total volume of 1X Diluent Solution required to dilute stock Mouse Complement C3 Calibrator to prepare **Standard #1**
V_T = Total volume of **Standard #1**
Calculate the dilution factor (D_F) between stock calibrator and the **Standard #1** final concentration:

$$Cs/C_F = D_F$$
$$2{,}740 / 200 = 13.7$$

Calculate the final volume V_D required to prepare the **Standard #1** at 200 ng/mL

$$V_A * D_F = V_T$$
$$V_D = V_T - V_A$$
$$60 * 13.7 = 822\ \mu L$$
$$VD = 822 – 60 = 762\ \mu L$$

To tube #1, add 60 µL of reconstituted Mouse Complement C3 Calibrator to 762 µL of 1X Diluent Solution to obtain a concentration at 200 ng/mL (**Standard #1**).

2.4 Add 300 µL 1X Diluent Solution into tube numbers 2 - 7.

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

2.6 Prepare **Standard #3** by adding 300 µL from **Standard #2** to #3. Mix thoroughly and gently.

2.7 Using the table below as a guide to prepare further serial dilutions.

2.8 1X Diluent Solution serves as the zero standard (0 ng/mL).

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See step 2.3				200
2	300	300	600	200	100
3	300	300	600	100	50
4	300	300	600	50	25
5	300	300	600	25	12.5
6	300	300	600	12.5	6.25
7	300	300	600	6.25	3.125

3. Sample Collection, Storage and Preparation

3.1 **Serum:** Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation.

3.2 **Plasma:** For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results.

Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Precautions
For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

Additives and Preservatives
No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

General Sample Preparation:
The assay for quantification of Complement C3 in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/50,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- To prepare a 1/50,000 dilution of sample, transfer 5 µL of sample to 995 µL of 1X diluent. This gives you a 1/200 dilution.
- Next, dilute the 1/200 samples by transferring 4 µL, to 996 µL of 1X diluent. You now have a 1/50,000 dilution of your sample.
- Mix thoroughly at each stage.

4. 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.

4.1 Pipette 100 µL of standards, including zero control, in duplicate, into pre designated wells.

4.2 Pipette 100 µL of sample (in duplicate) into pre designated wells.

4.3 Incubate the microtiter plate at room temperature for twenty (20 ± 2) minutes. Keep plate covered and level during incubation.

4.4 Following incubation, aspirate the contents of the wells.

4.5 Completely fill each well with appropriately diluted 1X Wash Buffer and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by gently striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

4.6 Pipette 100 µL of appropriately 1X Enzyme-Antibody Conjugate to each well. Incubate at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

4.7 Wash and blot the wells as described in 4.4 - 4.5.

4.8 Pipette 100 µL of TMB Substrate Solution into each well.

4.9 Incubate in the dark at room temperature for precisely ten (10) minutes.

4.10 After ten minutes, add 100 µL of Stop Solution to each well.

Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

Calculations:

Average the duplicate standard reading for each standard, sample and control blank. Subtract the control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in 1X Incubation Buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

Interferences:

These chemicals or biologicals will cause interferences in this assay causing compromised results or complete failure.

- Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)

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