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# ab108824
# Human Complement C4 ELISA Kit

For the quantitative measurement of human Complement C4 in plasma and serum.

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

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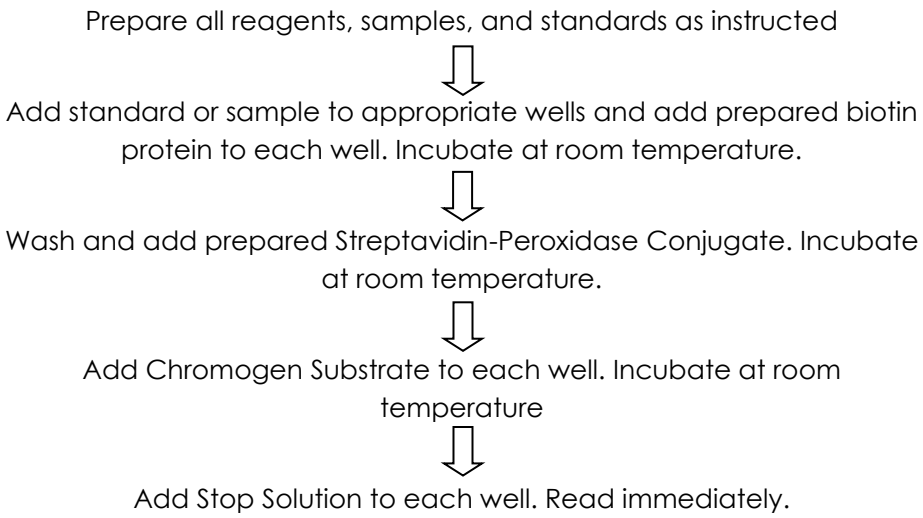
# 1. Overview

Complement C4 Human *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab108824) is designed for the quantitative measurement of Complement C4 levels in plasma and serum.

A Complement C4 specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently biotinylated Complement C4 is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is inversely proportional to the amount of Complement C4 captured in plate.

Complement component 4 (C4) plays a key role in the activation of the classical complement pathway. C4 is synthesized as a single-chain precursor molecule (200 kDa) but processed to the three-chain disulphide-linked structure with alpha (93 kDa), beta (78 kDa), and gamma (33 kDa) chains prior to secretion. After activation by C1s, C4 is processed to C4a and C4b. C4a anaphylatoxin is a mediator of local inflammation and induces smooth muscle contraction. C4b, the major activation product, is an essential subunit of the C3 and C5 convertases of the classical complement pathway. C4 deficiency is associated with systemic lupus erythematosus. The C4b degradation product C4d is a marker for humoral rejection in allografts.

## 2. Protocol Summary



## 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.
- 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

## 4. Storage and Stability

**Store kit at +4°C immediately upon receipt, apart from the SP Conjugate, which should be stored at -20°C.**

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 |
|---|---|---|
| Complement C4 Microplate (12 x 8 wells) | 96 wells | 4°C |
| Complement C4 Standard | 1 vial | 4°C |
| 10X Diluent N Concentrate | 30 mL | 4°C |
| Biotinylated Human Complement C4 (Lyophilized) | 1 vial | 4°C |
| 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) | 80 µL | -20°C |
| Chromogen Substrate | 7 mL | 4°C |
| Stop Solution | 11 mL | 4°C |
| 20X Wash Buffer Concentrate | 30 mL | 4°C |
| Sealing Tapes | 3 | N/A |

## 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 1 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.
- 6 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.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- 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.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

## 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 Diluent N

Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

### 9.2 1X Wash Buffer

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

### 9.3 2X Biotinylated Complement C4

9.3.1 Add 3 mL 1X Diluent N to the lyophilized Biotinylated Complement C4 vial to generate a 1X Biotinylated Complement C4 stock solution.

9.3.2 Allow the vial of 1X Biotinylated Complement C4 stock solution to sit for 10 minutes with gentle agitation prior to use. From the stock solution, dilute 2-fold with Diluent N to produce a 1x working solution.
*Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.*

### 9.4 1X SP Conjugate

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N.

*Any remaining solution should be frozen at -20°C.*

## 10. 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).

**10.1** Reconstitution of the Complement C4 Standard vial to prepare a 10 µg/mL Complement C4 **Stock Standard**.

10.1.1 First consult the Complement C4 Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the Complement C4 Standard vial to produce a 10 µg/mL Complement C4 **Standard stock** by using the following equation:

$C_S$ = Starting mass of Complement C4 Standard (see vial label) (µg)

$C_F$ = 10 µg/mL Complement C4 **Stock Standard** final required concentration

$V_D$ = Required volume of 1X Diluent N for reconstitution (µL)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

**NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.**

$C_S$ = 5.5 µg of Complement C4 Standard in vial

$C_F$ = 10 µg/mL Complement C4 **Stock Standard** final concentration

$V_D$ = Required volume of 1X Diluent N for reconstitution

(5.5 µg / 10 µg/mL) x 1,000 = 550 µL

10.1.3 First briefly centrifuge the Complement C4 Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the Complement C4 Standard vial by adding the appropriate calculated amount $V_D$ of 1X Diluent N to the vial to generate the 10 µg/mL Complement C4 **Stock Standard.** Mix gently and thoroughly.

**10.2** Allow the reconstituted 10 µg/mL Complement C4 **Stock Standard** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

**10.3** Label six tubes #1 – 6.

**10.4** Prepare the 10 µg/mL **Standard #1** by adding 240 µL of the reconstituted 10 µg/mL Complement C4 **Stock Standard** to vial #1.

**10.5** Add 120 µL of 1X Diluent N to tube #2 – 6.

**10.6** To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

**10.7** To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

**10.8** Using the table below as a guide, prepare subsequent serial dilutions.

**10.9** 1X Diluent N serves as the zero standard (0 ng/mL).

| Standard # | Volume to Dilute (µL) | Volume Diluent N (µL) | Total Volume (µL) | Starting Conc. (µg/mL) | Final Conc. (µg/mL) |
|---|---|---|---|---|---|
| 1 | Step 10.4 | | | | 10.000 |
| 2 | 120 | 120 | 240 | 10.000 | 5.000 |
| 3 | 120 | 120 | 240 | 5.000 | 2.500 |
| 4 | 120 | 120 | 240 | 2.500 | 1.250 |
| 5 | 120 | 120 | 240 | 1.250 | 0.625 |
| 6 | - | 120 | 120 | - | 0 |

# 11. Sample Preparation

## 11.1 Plasma:

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant (EDTA or Heparin can also be used as an anticoagulant). Centrifuge samples at 3,000 x g for 10 minutes. Samples are recommended for use at 1:200 into 1X Diluent N and assay. Depending on application needs, the user should determine proper dilutions. The undiluted samples should be aliquoted to limit repeated freeze-thaw cycles and stored at -80°C for up to 3 months. When needed, the frozen sample should be thawed rapidly in a water bath at 37°C and immediately placed on ice until use to prevent complement activation.

## 11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes and remove serum. Samples are recommended for use at 1:200 into 1X Diluent and assay. Depending on application needs, the user should determine proper dilutions. Avoid repeated freeze-thaw cycles.

***Refer to Dilution Guidelines for further instruction.***

| **Guidelines for Dilutions of 100-fold or Greater**<br>*(for reference only; please follow the insert for specific dilution suggested)* | |
|---|---|
| **100x** | **10000x** |
| 4 µl sample + 396 µl buffer (100X)<br>= 100-fold dilution<br><br>*Assuming the needed volume is less than or equal to 400 µl* | A) 4 µl sample + 396 µl buffer (100X)<br>B) 4 µl of A + 396 µl buffer (100X)<br>= 10000-fold dilution<br><br>*Assuming the needed volume is less than or equal to 400 µl* |
| **1000x** | **100000x** |
| A) 4 µl sample + 396 µl buffer (100X)<br>B) 24 µl of A + 216 µl buffer (10X)<br>= 1000-fold dilution<br><br>*Assuming the needed volume is less than or equal to 240 µl* | A) 4 µl sample + 396 µl buffer (100X)<br>B) 4 µl of A + 396 µl buffer (100X)<br>C) 24 µl of A + 216 µl buffer (10X)<br>= 100000-fold dilution<br><br>*Assuming the needed volume is less than or equal to 240 µl* |

## 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 plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

## 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** Prepare all reagents, working standards, and samples as directed in the previous sections. The assay is performed at room temperature (10-25°C).

**13.2** Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

**13.3** Add 25 µL of Complement C4 Standard or sample to each well and immediately add 25 µL of 1X Biotinylated Complement C4 to each well (on top of the standard or sample). Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for two hours at room temperature. Start the timer after the last sample addition.

**13.4** Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4 5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

**13.5** Add 50 µL of 1X SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

**13.6** Wash microplate as described above.

**13.7** Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 20 minutes or till the optimal blue color density develops.

**13.8** Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.

**13.9** Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at low concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

## 14. Calculations

Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution.

## 15. Typical Data

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

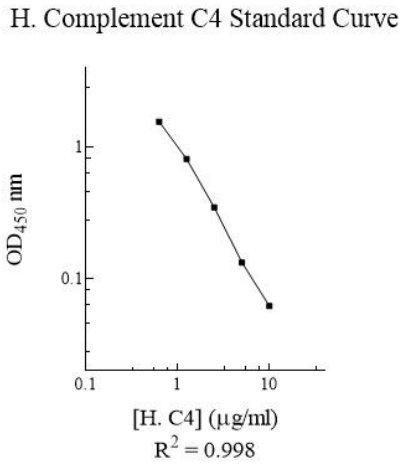



**Figure 1**. Example of Complement C4 standard curve. The standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

## 16. Typical Sample Values

**SENSITIVITY** –

The minimum detectable dose (MDD) of Complement C4 is typically ~0.5 µg/ml, as calculated by 2SD from the mean of a zero standard.

**PRECISION –**

| | Intra-assay Precision | Inter-Assay Precision |
|---|---|---|
| CV (%) | 3.1 | 10.5 |

**REFERENCE VALUE –**

Average normal human complement C4 plasma and serum levels value is 160 – 480 µg/mL

**SPIKING RECOVERY** –

Recovery was determined by spiking two plasma samples with different complement C4 concentrations.

| Sample | Unspiked Sample (µg/mL) | Spiking Value (µg/mL) | Recovery (%) |
|---|---|---|---|
| Plasma | 1.076 | 1.949 | 92 |
| | | 1.279 | 83 |
| | | 0.554 | 95 |
| Serum | 2.181 | 1.949 | 99 |
| | | 1.279 | 81 |
| | | 0.554 | 90 |
| **Average Recovery (%)** | | | 90% |

**LINEARITY OF DILUTION -**

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Plasma and serum samples were serially-diluted to test for linearity.

| **Average Percentage of Expected Value (%)** | | |
|---|---|---|
| Dilution Factor | Plasma | Serum |
| 1:100 | 90 | 91 |
| 1:200 | 101 | 96 |
| 1:400 | 109 | 110 |

## 17. Assay Specificity

This kit recognizes Complement C4 in serum and plasma.

| Species | Cross-Reactivity (%) |
|---|---|
| Canine | None |
| Bovine | None |
| Equine | <3% |
| Monkey | None |
| Mouse | None |
| Rat | None |
| Swine | None |
| Rabbit | None |
| **Protein** | **Cross-Reactivity (%)** |
| Human Complement C2 | <5% |
| Human Complement C3 | <3% |
| Human Complement Factor H | <2% |
| Human Complement Factor I | <5% |
| Human Complement Factor P | <5% |

**INTERFERENCES –**

No significant cross-reactivity observed with human complement C1, C5, C6, C7, C8, C9, Factor B, and Factor D proteins.

## 18. Troubleshooting

| Problem | Cause | Solution |
|---|---|---|
| Poor standard curve | Improper standard dilution | Confirm dilutions made correctly |
| | Standard improperly reconstituted (if applicable) | Briefly spin vial before opening; thoroughly resuspend powder (if applicable) |
| | Standard degraded | Store sample as recommended |
| | Curve doesn't fit scale | Try plotting using different scale |
| Low signal | Incubation time too short | Try overnight incubation at 4°C |
| | Target present below detection limits of assay | Decrease dilution factor; concentrate samples |
| | Precipitate can form in wells upon substrate addition when concentration of target is too high | Increase dilution factor of sample |
| | Using incompatible sample type (e.g. serum vs. cell extract) | Detection may be reduced or absent in untested sample types |
| | Sample prepared incorrectly | Ensure proper sample preparation/dilution |

| Problem | Cause | Solution |
|---|---|---|
| Large CV | Bubbles in wells | Ensure no bubbles present prior to reading plate |
| | All wells not washed equally/thoroughly | Check that all ports of plate washer are unobstructed wash wells as recommended |
| | Incomplete reagent mixing | Ensure all reagents/master mixes are mixed thoroughly |
| | Inconsistent pipetting | Use calibrated pipettes and ensure accurate pipetting |
| | Inconsistent sample preparation or storage | Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles) |

| Problem | Cause | Solution |
|---|---|---|
| High background/ Low sensitivity | Wells are insufficiently washed | Wash wells as per protocol recommendations |
| | Contaminated wash buffer | Make fresh wash buffer |
| | Waiting too long to read plate after adding STOP solution | Read plate immediately after adding STOP solution |
| | Improper storage of ELISA kit | Store all reagents as recommended. |
| | Using incompatible sample type (e.g. Serum vs. cell extract) | Detection may be reduced or absent in untested sample types |

## 19. Notes

**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)