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ab222866 Human Complement C4-Binding Protein ELISA Kit

For the quantitative measurement of human Complement C4-Binding Protein in plasma, serum, urine, milk, saliva and cerebrospinal fluid samples.

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

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1. Overview

ab222866 Human Complement C4-Binding Protein ELISA Kit is designed for the quantitative measurement of Complement C4-Binding Protein in plasma, serum, urine, milk, saliva and cerebrospinal fluid (CSF) samples.

The kit employs a quantitative sandwich enzyme immunoassay technique that measures human Complement C4-Binding Protein (C4BP) in less than 4 hours. A polyclonal antibody specific for human complement C4BP has been pre-coated onto a 96-well microplate with removable strips. Complement C4BP in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for complement C4BP, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Complement component C4-binding protein (C4BP) regulates the complement system by accelerating the decay of the complement component C3 convertase and by acting as a cofactor to the serine protease factor I in the degradation of C4b. C4BP is a high molecular mass (570 kDa) glycoprotein and is present in plasma in various isoforms with different alpha beta composition. The major form of C4BP is composed of seven identical 70-kDa alpha chains, each containing a binding site for the complement protein C4b, and a unique 45 kDa beta chain which contains a binding site for the vitamin K-dependent protein S. C4BP was overexpressed in the synovial membranes of patients with rheumatoid arthritis. It was detected in amyloid-beta plaques and on apoptotic cells.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells. Incubate at room temperature for 2 hours

Add 50 µL Biotinylated Antibody to all wells. Incubate at room temperature for 1 hour

Wash wells. Add 50 µL Streptavidin-Peroxidase Conjugate to all wells. Incubate at room temperature for 30 minutes

Wash wells. Add 50 µL Chromogen Substrate to all wells. Incubate at room temperature for 20 minutes

Add 50 µL Stop Solution and read OD at 450 nm

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 handle 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 4°C immediately upon receipt, apart from the Streptavidin-Peroxidase Conjugate and Biotinylated Antibody, which should be stored at -20°C. 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
Anti- Human Complement C4BP coated Microplate (12 x 8 wells)	96 wells	+4°C
Human Complement C4BP Standard	1 Vial	+4°C
Biotinylated Human Complement C4BP	120 µL	-20°C
10X Diluent N Concentrate	30 mL	+4°C
20X Wash Buffer Concentrate	2 x 30 mL	+4°C
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
Chromogen Substrate	7 mL	-+4°C
Stop Solution	11 mL	+4°C
Sealing Tapes	3	+4°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.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.

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:

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Store for up to 30 days at +4°C.

9.2 Biotinylated Human Complement C4BP:

Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with 1X Diluent N. Any remaining solution should be frozen at -20°C.

9.3 1X Wash Buffer Concentrate:

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the 20X Wash Buffer Concentrate with reagent grade water.

9.4 1X Streptavidin-Peroxidase Conjugate:

Spin down the Streptavidin-Peroxidase 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.

9.5 Anti-Human CD300A coated Microplate (12 x 8 wells):

Ready to use. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

9.6 Chromagen Substrate:

Ready to use. Store at +4°C.

9.7 Sealing Tapes:

Ready to use. Store at +4°C.

9.8 Stop Solution:

Ready to use. Store at +4°C.

9.9 Human C4BP Standard:

Reconstitute the 60 ng of Human Complement C4BP Standard with 1 mL of 1X Diluent N to generate a 60 ng/mL standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions.

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 Reconstitute the C4BP Stock to generate a 60 ng/mL **Standard #1**.

10.1.1 First consult the C4BP 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 C4BP Standard vial to produce a 60 ng/mL C4BP Standard stock by using the following equation:

CS = Starting mass of C4BP Standard stock (see vial label) (ng)

CF = 60 ng/mL C4BP Standard #1 final required concentration

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

Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_S) * 1{,}000 = V_D$$

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 = 60 ng of C4BP Standard in vial

C_F = 60 ng/mL C4BP **Standard #1** final concentration

V_D = Required volume of 1X Diluent N for reconstitution

(60 ng / 60 ng/mL) * 1,000 = 1,000 µL

10.1.3 First briefly centrifuge the C4BP Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the C4BP Standard vial by adding the appropriate calculated amount VD of 1X Diluent N to the vial to generate the 60 ng/mL C4BP **Standard #1.** Mix gently and thoroughly.

10.2 Allow the reconstituted 60 ng/mL C4BP **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label seven tubes #2 – 8.

10.4 Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (60 ng/mL) 1:2 with 1X Diluent N to produce 30, 15, 7.5, 3.75, 1.875, and 0.938 ng/mL solutions. 1X Diluent N serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and used within 30 days

10.5 Add 120 µL of 1X Diluent N to tube #2 – 8.

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.

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	C4BP (ng/mL)
1	Step 10.1		60.0
2	120 µL Standard #1	120	30.0
3	120 µL Standard #2	120	15.0
4	120 µL Standard #3	120	7.50
5	120 µL Standard #4	120	3.750
6	120 µL Standard #5	120	1.875
7	120 µL Standard #6	120	0.938
8 (Blank)	N/A	120	0

11. Sample Preparation

11.1 Plasma:

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Plasma dilution is suggested at 1:40000 into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. Serum dilution is suggested at 1:40000 into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. 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.

11.3 Urine:

Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Urine dilution is suggested at 1:2 into 1X Diluent N or within a range of 1X – 100X; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.4 Saliva:

Collect saliva using sample pot. Centrifuge samples at 800 x g for 10 minutes. Saliva dilution is suggested at 1:80 into 1X Diluent N or within a range of 8X – 800X; however, user should determine the optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.5 Milk:

Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Milk dilution is suggested at 1:800 into 1X Diluent N or within a range of 80X – 8000X; however, the user should

determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.6 CSF:

Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. CSF dilution is suggested at 1:20 into 1X Diluent N or within a range of 5X – 2000X; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

12. 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.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.3 Add 50 µL of Human Complement C4BP Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

12.4 Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.

12.5 Add 50 µL of Biotinylated Human Complement C4BP Antibody to each well and incubate for 1 hour.

12.6 Wash the microplate as described above (Step 12.4).

12.7 Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes.

12.8 Turn on the microplate reader and set up the program in advance.

12.9 Wash the microplate as described above (Step 12.4).

12.10 Add 50 µL of Chromogen Substrate per well and incubate for 20 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

12.11 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.

12.12 Read the absorbance on the 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.
∆ **Note:** Some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

13. Calculations

13.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

13.2 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 four-parameter or log-log logistic curve-fit.

13.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

14. Typical Data

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

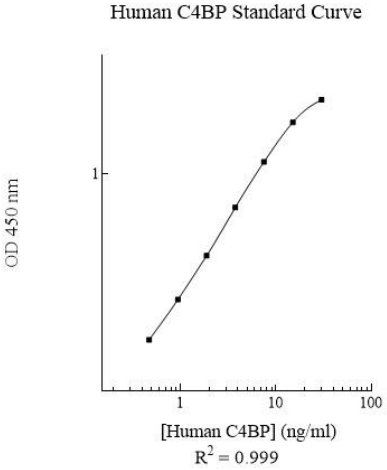

Figure 1. Example of human Complement C4-Binding Protein standard curve.

15. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of complement C4BP is typically 0.14 ng/mL.

PRECISION –

	Intra-Assay	Inter-Assay
% CV	4.7	10.3

RECOVERY –

Standard Added Value	1.875 – 15 ng/mL
Recovery %	87 – 113%
Average Recovery %	96%

Linearity of Dilution

Average Percentage of Expected Value (%)		
Sample Dilution	**Plasma**	**Serum**
20000	92%	91%
40000	98%	98%
80000	107%	106%

16. Assay Specificity

This kit recognizes human complement C4BP in plasma, serum, urine, milk, saliva and CSF samples.

CROSS REACTIVITY

Proteins	Cross Reactivity (%)
Complement C1	None
Complement C3	None
Complement C4BP	100%
Complement C4	None
Complement C5	None
Complement C6	None
Complement C7	None
Complement C8	None
Complement C9	None

17. Species Reactivity

This kit recognizes human complement C4BP.

Species	Cross Reactivity (%)
Monkey	None
Mouse	None
Rat	None
Swine	None
Canine	None
Bovine	None
Human	100

REFERENCE VALUE -

On average, normal human C4BP plasma level is 200 µg/mL.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are dry after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing

Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.
Deficient Standard Curve fit	Non-optimal sample dilution	Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples.
	Contamination of reagents.	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)