ab195461 –

Complement C3b Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Complement C3b in plasma, serum, milk, urine, saliva, and CSF samples.

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

Version 11a Last Updated 6 November 2025

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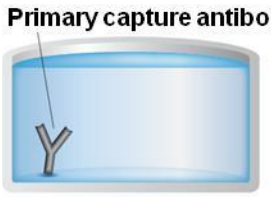
1. BACKGROUND

Abcam's Complement C3b Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Complement C3b concentrations in Human plasma, serum, milk, urine, saliva, and CSF samples.

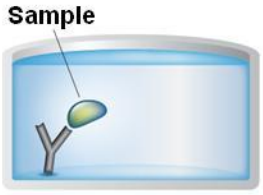
A Complement C3b specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently a Complement C3b specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate 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 directly proportional to the amount of Complement C3b captured in plate.

Complement component 3 (C3) plays a central role in all three complement activation pathways. The C3 precursor contains 1,663 amino acids and has a molecular weight of about 180 kDa. Human C3 has 77% identity to mouse C3 at the amino acid level. C3 is cleaved by C3 convertase into two activated fragments C3a and C3b. The anaphylatoxin C3a is a vasoactive peptide and a mediator of local inflammatory process. The C3b in complex with receptor can bind covalently to pathogen surfaces to promote phagocytosis. Acquired C3 deficiency is associated with severe recurrent meningococcal and pneumococcal infections. Plasma C3 and C3a levels are elevated in cryptogenic and large-vessel disease subtypes of ischemic stroke.

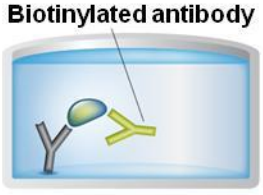
2. ASSAY SUMMARY



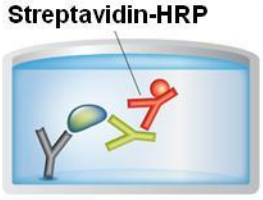
Prepare all reagents, samples and standards as instructed.



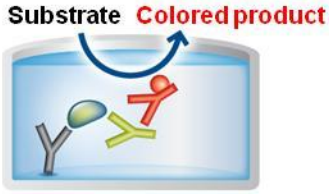
Add standard or sample to each well used. Incubate at room temperature.



Wash and add prepared biotin antibody to each well. Incubate at room temperature.



Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.



Add Chromogen Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read 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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Upon arrival, immediately store components of the kit at 2-8ºC, apart from the SP Conjugate & Biotinylated Antibody, 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 section 12. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Human Complement C3b Microplate (12 x 8 well strips)	96 wells	4°C
Human Complement C3b Standard (Lyophilized)	1 vial	4°C
10X Diluent M Concentrate	30 mL	4°C
Biotinylated Complement C3b Antibody	1 vial	-20°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	2 x 30 mL	4°C
Sealing Tapes	3	N/A

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- 1 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.
- 8 tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- 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.
- Complete removal of all solutions and buffers during wash steps.
- **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.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

9.1 **1X Diluent M**

Dilute the 10X Diluent M 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 **1X Biotinylated Complement C3b Detector Antibody**

9.3.1 The stock Biotinylated Complement C3b Antibody must be diluted with 1X Diluent M according to the label concentration to prepare 1X Biotinylated Complement C3b Detector Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated Complement C3b Antibody.

9.3.2 Calculate the necessary amount of 1X Diluent M to dilute the Biotinylated Complement C3b Antibody to prepare a 1X Biotinylated Complement C3b Detector Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation**. Please note the volumes below do not account for an overage for pipette losses:**

Number of Wells Strips	Number of Wells	(V_T) Total Volume of 1X Biotinylated Detector Antibody (µL)
4	32	1,600
6	48	2,400
8	64	3,200
10	80	4,000
12	96	4,800

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

Where:

C_S = Starting concentration (X) of stock Biotinylated Complement C3b Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated Complement C3b Detector Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated Complement C3b Detector Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated Complement C3b Antibody

V_D = Total volume of 1X Diluent M required to dilute (X) stock Biotinylated Complement C3b Antibody to prepare 1X Biotinylated Detector Antibody solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent M required to prepare the 1X Biotinylated Complement C3b Antibody:

$$V_T - V_A = V_D$$

Example:

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

C_S = 50X Biotinylated Complement C3b Antibody stock

C_F = 1X Biotinylated Complement C3b Detector Antibody solution for use in the assay procedure

V_T = 3,200 µL (8 well strips or 64 wells)

$$(1X/50X) \times 3{,}200\ \mu L = 64\ \mu L$$

$$3{,}200\ \mu L - 64\ \mu L = 3{,}136\ \mu L$$

V_A = 64 µL total volume of (X) stock Biotinylated Complement C3b Antibody required

V_D = 3,136 µL total volume of 1X Diluent M required to dilute the 50X stock Biotinylated Antibody to prepare 1X Biotinylated

Complement C3b Detector Antibody solution for assay procedures

9.3.3 First centrifuge the Biotinylated Complement C3b Antibody vial to collect the contents at the bottom.

9.3.4 Add calculated amount V_A of stock Biotinylated Complement C3b Antibody to the calculated amount V_D of 1X Assay Diluent M. Mix gently and thoroughly.

9.4 **1X SP Conjugate**

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

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

10. STANDARD PREPARATIONS

- **Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.**
- **Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.**
- **This procedure prepares sufficient standard dilutions for duplicate wells.**

10.1 Reconstitution of the stock Complement C3b Standard vial to prepare a 5 ng/mL Complement C3b **Standard #1**:

10.1.1 First consult the Complement C3b Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent M to add when resuspending the Complement C3b Standard vial to produce a 5 ng/mL Complement C3b Standard stock by using the following equation:

C_S = Starting mass of Complement C3b Standard stock (see vial label) (ng)
C_F = 5 ng/mL Complement C3b **Standard #1** final required concentration
V_D = Required volume of 1X Diluent M 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 = 9 ng of Complement C3b Standard in vial

C_F = 5 ng/mL Complement C3b **Standard #1** final concentration

V_D = Required volume of 1X Diluent M for reconstitution

(9 ng / 5 ng/mL) * 1,000 = 1,800 µL

10.1.3 First briefly centrifuge the Complement C3b Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the Complement C3b Standard vial by adding the appropriate calculated amount V_D of 1X Diluent M to the vial to generate the 5 ng/mL Complement C3b **Standard #1**. Mix gently and thoroughly.

10.2 Allow the reconstituted 5 ng/mL Complement C3b **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label seven tubes #2 – 8.

10.4 Add 120 µL of 1X Diluent M to tube #2 – 8.

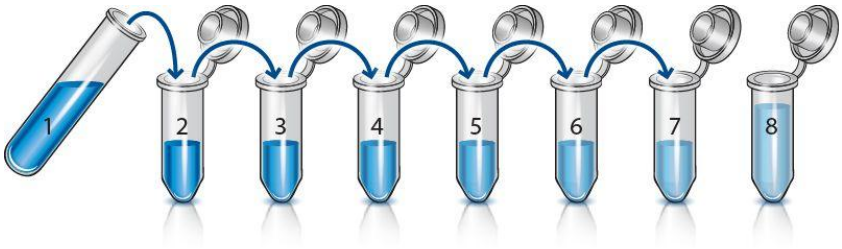
10.5 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

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

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

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Volume Diluent M (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Step 10.1				5.000
2	120	120	240	5.000	2.500
3	120	120	240	2.500	1.250
4	120	120	240	1.250	0.625
5	120	120	240	0.625	0.313
6	120	120	240	0.313	0.156
7 (Blank)	-	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 3000 x *g* for 10 minutes. A 1:800,000 dilution into 1X Diluent M to assay is suggested; 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.

11.2 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. A 1:800,000 dilution into 1X Diluent M to assay is suggested; 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 -20°C or below for up to 3 months.

11.3 Urine

Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. A 4-fold sample dilution is suggested into Diluent M or within the range of 1x – 8x; 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. A 1:400 dilution into 1X Diluent M to assay is suggested; 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.

11.5 **Milk**

Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. A 1:8000 sample dilution is suggested into Diluent M or within the range of 2000x – 40000x; 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.

11.6 **CSF**

Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. A 1:8000 sample dilution is suggested into Diluent M or within the range of 50x – 50000x; 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. PLATE PREPARATION

- The 96 well plate strips included with this kit is 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).
- 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.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (20-25°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 50 µL of standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

13.4 Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, 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 Biotinylated Complement C3b Detector Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for one hour.

13.6 Wash microplate as described above.

13.7 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. Incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

13.8 Wash microplate as described above.

13.9 Add 50 µL of Chromogen Substrate per well and incubate in ambient light for 30 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.

13.10 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.

13.11 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 high 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 (OD) 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 factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

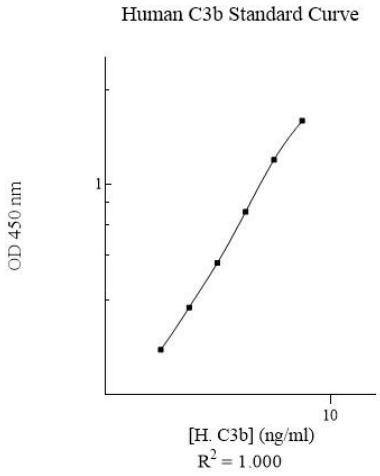


Figure 1. Example of Complement C3b standard curve prepared as described in Section 10.

16. TYPICAL SAMPLE VALUES

SENSITIVITY -

The minimum detectable dose of Human Complement C3b as calculated by 2SD from the mean of a zero standard was established to be 67 pg/ml.

Intra-assay precision was determined by testing three plasma samples twenty times in one assay.

Inter-assay precision was determined by testing three plasma samples in twenty assays.

SPIKING RECOVERY –

Recovery was determined by spiking one plasma and one serum sample with different human complement C3b concentrations

Sample	Unspiked Sample (ng/ml)	Spiking Value (ng/ml)	Expected	Observed	Recovery (%)
Plasma	1.074	1.108	2.182	2.382	109
		0.576	1.650	1.513	92
		0.296	1.370	1.468	107
Serum	0.924	1.108	2.032	2.163	106
		0.576	1.5	1.571	105
		0.296	1.22	1.306	107
Average Recovery (%)					**104**

LINEARITY OF DILUTION -

Plasma Dilution	Average % Expected Value
1:400,000	96
1:800,000	98
1:1600,000	106

Serum Dilution	Average % Expected Value
1:200,000	96
1:400,000	107
1:800,000	98

PRECISION –

	Intra- Assay	Inter- Assay
% CV	4.0	9.6

REFERENCE VALUE –

Normal human complement C3b plasma levels range from 300 – 1600 µg/ml.

17. ASSAY SPECIFICITY

Species	% Cross Reactivity
Canine	None
Bovine	None
Equine	None
Monkey	50
Mouse	None
Rat	None
Swine	None
Human	100
Protein	**% Cross Reactivity**
Human Complement C1	5
Human Complement C3	10
Human Complement C3c	10
Human Complement C8	3

No significant cross-reactivity observed with human complement C1q, C1r, C1s, C2, C3d, C4, C4BP, C5, C6, C7, C8G, C9, factor B, factor D, factor H, factor I, and factor P proteins.

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

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)