ab195460 –

Human Complement Factor I ELISA Kit

Instructions for Use

For the quantitative measurement of Human Complement Factor I in plasma, serum, milk, saliva, and cell culture supernatants.

View kit datasheet: www.abcam.com/ab195460
(use www.abcam.cn/ab195460 for China, or www.abcam.co.jp/ab195460 for Japan)

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

Version 3a Last Updated 26 April 2023

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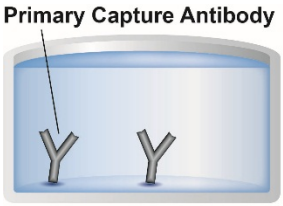
1. BACKGROUND

Abcam's Human Complement Factor I *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Complement Factor I levels in plasma, serum, milk, saliva, and cell culture supernatant.

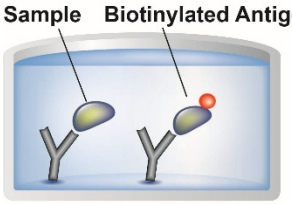
A Complement Factor I specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently biotinylated Complement Factor I is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate is added and unbound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

Complement Factor I (FI), known as C3b/C4b inactivator, is a glycosylated plasma serine proteinase of complement regulatory enzyme. FI is synthesized as a 583-residue single-chain precursor (88 kDa) which is processed into a heterodimer consisting of disulfide-linked heavy (50 kDa) and light (38 kDa) chains. It circulates in an inactive zymogen-like state despite being fully processed to the 321-residue mature protein. In the presence of additional regulatory cofactors such as C4b-binding protein, factor H, complement receptor 1, and membrane cofactor protein, FI can cleave and inactivate the complement components C3b and C4b to regulate the levels of C3 convertases. FI deficiency is associated with atypical hemolytic uraemic syndrome, primary glomerulonephritis, and recurrent pyogenic infections.

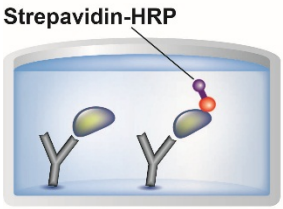
2. ASSAY SUMMARY



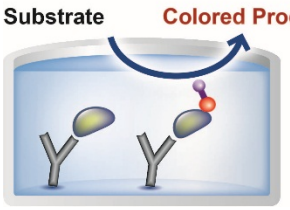
Prepare all reagents, samples and standards as instructed.



Add standard or sample to each well used and add prepared biotin protein to each well. Incubate at room temperature.



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



Wash and 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

Store kit at 2-8ºC 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 section 12. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Complement Factor I Microplate (12 x 8 well strips)	96 wells	2-8°C
Complement Factor I Standard (Lyophilized)	1 vial	2-8°C
10X Diluent M Concentrate	30 mL	2-8°C
Biotinylated Complement Factor I (Lyophilized)	1 vial	2-8°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	2-8°C
Stop Solution	11 mL	2-8°C
20X Wash Buffer Concentrate	30 mL	2-8°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 2 - 8°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 Factor I**

Add 4 mL 1X Diluent M to the lyophilised Biotinylated Complement Factor I vial to generate 1X Biotinylated Complement Factor I. Allow the vial of 1X Biotinylated Complement Factor I to sit for 10 minutes with gentle agitation prior to use.

Any remaining solution should be frozen at -20°C and used within 30 days.

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 Factor I Standard vial to prepare a 24 µg/mL Complement Factor I **Standard #1**:

10.1.1 First consult the Complement Factor I 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 Factor I Standard vial to produce a 24 µg/mL Complement Factor I Standard stock by using the following equation:

C_S = Starting mass of Complement Factor I Standard stock (see vial label) (ng)
C_F = 24 µg/mL Complement Factor I **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:

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

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 = 24 µg of Complement Factor I Standard in vial

C_F = 24 µg/mL Complement Factor I **Standard #1** final concentration

V_D = Required volume of 1X Diluent M for reconstitution

(24 µg / 24 µg/mL) x 1,000 = 1,000 µL

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

10.1.4 Reconstitute the Complement Factor I Standard vial by adding the appropriate calculated amount V_D of 1X Diluent M to the vial to generate the 24 µg/mL Complement Factor I **Standard #1**. Mix gently and thoroughly.

10.2 Allow the reconstituted 24 µg/mL Complement Factor I **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. (µg/mL)	Final Conc. (µg/mL)
1	Step 10.1				24.00
2	120	120	240	24.00	12.00
3	120	120	240	12.00	6.000
4	120	120	240	6.000	3.00
5	120	120	240	3.00	1.500
6	120	120	240	1.500	0.750
7	120	120	240	0.750	0.375
8 (Blank)	-	120	120	-	0

11. SAMPLE PREPARATION

11.1 Cell culture supernatants

Centrifuge cell culture media at 1500 rpm for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -80°C. Avoid repeated freeze-thaw cycles.

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. Dilute samples 1:20 into 1X Diluent M and assay. 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 Plasma

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x *g* for 10 minutes. Dilute samples 1:20 into 1X Diluent M. 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).

11.4 Saliva

Collect Human saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute saliva 1:2 into 1X Diluent M and assay. The samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.5 Milk

Collect Human milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. The samples can be stored at -20°C or below 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 (20-30°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 Factor I Standard or sample per well and immediately add 25 µL of 1X Biotinylated Complement Factor I to each well (on top of the sample or standard). 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 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 per well and incubate in ambient light for 10 minutes or until the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

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

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 factor.

15. TYPICAL DATA

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

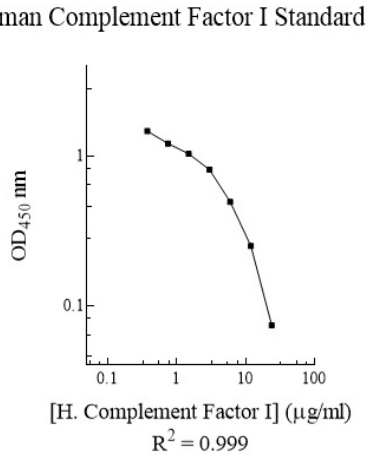


Figure 1. Example of a Complement Factor I standard curve prepared as described in Section 10.

16. TYPICAL SAMPLE VALUES

SENSITIVITY -

The minimum detectable dose of Human Complement Factor I is typically ~ 0.26 µg/mL.

RECOVERY –

Standard Added Value: 0.75 - 6 µg/ml

Recovery %: 89 - 111

Average Recovery %: 98

LINEARITY OF DILUTION -

Plasma Dilution	Average % Expected Value
1:10	104
1:20	101
1:40	93

Serum Dilution	Average % Expected Value
1:10	106
1:20	98
1:40	96

PRECISION –

	Intra-Assay	Inter-Assay
% CV	5.4	10

17. ASSAY SPECIFICITY

Species	% Cross Reactivity
Canine	None
Bovine	None
Equine	2
Monkey	None
Mouse	None
Rat	5
Swine	75
Human	None
Protein	**% Cross Reactivity**
Complement C1	None
Complement C2	None
Complement C3	None
Complement C4	None
Complement C5	None
Complement C6	None
Complement C7	None
Complement C8	None
Complement C9	None
Complement Factor B	None
Complement Factor D	None
Complement Factor H	None
Complement Factor P	None
Complement Factor I	100

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:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)