Version 13h Last updated 19 December 2025

ab108822
Human Complement C3 ELISA Kit

For the quantitative measurement of human Complement C3 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 C3 Human in vitro competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Complement C3 in Human plasma and serum.

A Complement C3 specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently a Complement C3 specific biotinylated detection protein 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 C3 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. Protocol Summary

Prepare all reagents, samples, and standards as instructed

⇩

Add standard, sample and prepared biotin protein to appropriate wells. 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.
- 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 handled 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 SP Conjugate, 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
Complement C3 Microplate (12 x 8 wells)	96 wells	4°C
Complement C3 Standard (Lyophilized)	1 vial	4°C
10X Diluent M Concentrate	30 mL	4°C
Biotinylated human Complement C3 (Lyophilized)	1 vial	4°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	1 vial	4°C
Stop Solution	1 vial	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.
- When diluting the concentrates, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution 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.

Δ Note: 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 C3

9.3.1 Add 3 mL 1X Diluent M to the lyophilized Biotinylated Complement C3 vial to generate a 1X Biotinylated Complement C3 stock solution.

9.3.2 Allow the vial of 1X Biotinylated Complement C3 stock solution to sit for 10 minutes with gentle agitation prior to use.
Δ Note: Any remaining solution should be frozen at -20°C.
Δ Note: 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 M.

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

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent and use within 30 days.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the Complement C3 Standard vial to generate a 7.5 µg/mL **Standard #1**.

10.1.1 First consult the Complement C3 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 C3 Standard vial to produce a 7.5 µg/mL Complement C3 **Standard #1** by using the following equation:

10.1.3

C_S = Starting mass of Complement C3 Standard (see vial label) (µg)

C_F = The 7.5 µg/mL Complement C3 **Standard #1** final required concentration

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

<u>Calculate total required volume 1X Diluent M for resuspension:</u>

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

<u>Example:</u>

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

C_S = 8.25 µg of Complement C3 Standard in vial

C_F = 7.5 µg/mL Complement C3 Standard final concentration

V_D = Required volume of 1X Diluent M for reconstitution

$$(8.25\ \mu g / 7.5\ \mu g/mL) \times 1{,}000 = 1{,}100\ \mu L$$

10.1.4 First briefly centrifuge the Complement C3 Standard Vial to collect the contents on the bottom of the tube.

10.1.5 Reconstitute the Complement C3 Standard vial by adding the appropriate calculated amount V_D of 1X Diluent M to the vial to generate the 7.5 µg/mL Complement C3 **Standard #1.** Mix gently and thoroughly.

10.2 Allow the reconstituted 7.5 µg/mL Complement C3 **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.

10.8 1X Diluent M serves as the zero standard (0 µg/mL).

Standard #	**Volume to dilute (µL)**	**Volume Diluent M (µL)**	**Human Complement C3 (µg/mL)**
1	Step 10.1		7.5
2	120 µL Standard #1	120	3.75
3	120 µL Standard #2	120	1.875
4	120 µL Standard #3	120	0.938
5	120 µL Standard #4	120	0.469
6	120 µL Standard #5	120	0.234
7	120 µL Standard #6	120	0.117
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 3,000 x g for 10 minutes and collect plasma. Dilute samples 1:800 into 1X Diluent M and assay. 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 3,000 x g for 10 minutes and remove serum. Dilute samples 1:800 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.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *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).
- Well effects have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- 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 (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 25 µL of Complement C3 Standard or sample per well and immediately add 25 µL of 1X Biotinylated Complement C3 to each well (on top of standard or sample). 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 Incubate in ambient light for 30 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 high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

13.10 Analyze the data as described below.

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

13.10.2 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.

13.10.3 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.

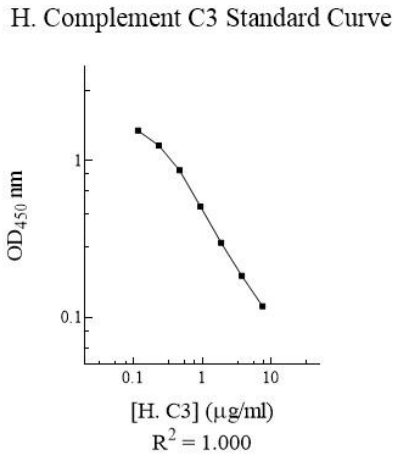

Figure 1. Example of Complement C3 standard curve. The standard curve was prepared as described in Section 10. Raw data values are shown in the table.

15. Typical Sample Values

SENSITIVITY –

The minimum detectable dose (MDD) of Complement C3 as calculated by 2 standard deviations from the mean of a zero standard was established to be 96 ng/mL.

PRECISION –

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

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

	Intra-assay Precision	**Inter-Assay Precision**
CV (%)	5.2	10.2

SPIKING RECOVERY –

Recovery was determined by spiking one plasma and one serum sample with different complement C3 concentrations.

Sample	**Unspiked Sample (µg/mL)**	**Spiking Sample (µg/mL)**	**Recovery (%)**
Recovery (%)	0.591	3.529	111
		0.847	107
		0.212	109
Average Recovery (%)	1.342	3.529	108
		0.847	105 101%
		0.212	93
Average Recovery (%)			105

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
1:400	90	101
1:800	102	98
1:1600	107	104

16. Assay Specificity

This kit recognizes Complement C3 in serum and plasma.

No significant cross-reactivity observed with complement C1, C1q, C1r, C1s, C2, C3c, C4, C4BP, C5, C6, C7, C8, C8G, C9, factor B, factor D, factor H, factor I, and factor P.

17. Species Reactivity

Species	% Cross Reactivity
Canine	< 4
Bovine	None
Equine	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Rabbit	None
Human	100
Protein	**% Cross Reactivity**
Complement C3b	60
Complement C3d	<2

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
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 (eg. 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. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

19. Notes

Technical Support

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