ab108823 - Complement C3 Human ELISA Kit

For the quantitative measurement of human Complement C3 in urine, milk, saliva, cerebrospinal fluid, tissue, cell lysates and cell culture supernatants.
This product is for research use only and is not intended for diagnostic use.

Storage and Stability

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

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 N Concentrate	30 mL	4°C
Biotinylated Human Complement C3 Antibody (50x)	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

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to 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.

Reagent Preparation

- Equilibrate reagents to room temperature. The kit contains enough reagents for 96 wells.
- Prepare fresh reagents immediately prior to use. When diluting the concentrates, make sure to rinse the bottle to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

1X Diluent N: Dilute the Diluent N Concentrate 1:10 with reagent grade water to produce a 1x solution. When diluting the concentrate, 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. Store for up to 30 days at 2-8°C

1X Wash Buffer: Dilute the Wash Buffer Concentrate 1:20 with reagent grade water to produce a 1x solution. When diluting the concentrate, 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.

1X Biotinylated Complement C3 Detector Antibody

- The stock Biotinylated Complement C3 Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated Complement C3 Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated Complement C3 Antibody. **NOTE**: The "X" concentration on the vial is lot dependent, so it is important to check this with each opened kit.
- Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated Complement C3 Antibody to prepare a 1X Biotinylated Complement C3 Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation. **Please note that 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 Antibody (µL)
4	32	1,600
6	48	2,400
8	64	3,200
10	80	4,000
12	96	4,800

Where:

C_S = Starting concentration (X) of stock Biotinylated Complement C3 Antibody (variable)
C_F = Final concentration (always = 1X) of 1X Biotinylated Complement C3 Antibody solution for the assay procedure
V_T = Total required volume of 1X Biotinylated Complement C3 Antibody solution for the assay procedure
V_A = Total volume of (X) stock Biotinylated Complement C3 Antibody
V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated Complement C3 Antibody to prepare 1X Biotinylated Complement C3 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 N required to prepare the 1X Biotinylated Complement C3 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 C3 Antibody stock
C_F = 1X Biotinylated Complement C3 Antibody solution for use in the assay procedure
V_T = 3,200 µL (8 well strips or 64 wells)

(1X/50X) x 3,200 µL = 64 µL
3,200 µL - 64 µL = 3,136 µL

V_A = 64 µL total volume of (X) stock Biotinylated Complement C3 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 C3 Antibody solution for assay procedures.

First spin the Biotinylated Complement C3 Antibody vial to collect the contents at the bottom.

Add calculated amount VA of stock Biotinylated Complement C3 Antibody to the calculated amount VD of 1X Assay Diluent N. Mix gently and thoroughly.

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.

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

Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Stored at -20°C after reconstitution & use within 30 days.

The preparation of a standard curve for duplicate measurements (recommended):

- Reconstitute the Complement C3 Standard vial to generate a 40 ng/mL Standard #1.

First consult the Complement C3 Standard vial to determine the mass of protein in the vial. Calculate the appropriate volume of 1X Diluent N to add when resuspending the Complement C3 Standard vial to produce a 40 ng/mL Complement C3 Standard stock by using the following equation:

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

C_F = 40 ng/mL Complement C3 **Standard #1** 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 = 72 ng of Complement C3 Standard in vial

C_F = 40 ng/mL Complement C3 **Standard #1** final concentration

V_D = Required volume of 1X Diluent N for reconstitution

(72 ng / 40 ng/mL) x 1,000 = 1800 µL

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

Reconstitute the Complement C3 Standard vial by adding the appropriate calculated amount VD of 1X Diluent N to the vial to generate the 40 ng/mL Complement C3 **Standard #1**. **Mix gently and thoroughly.**

Allow the reconstituted 40 ng/mL Complement C3 Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

Label seven tubes #2 – 8.

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

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

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

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

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

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	Human Complement C3 (ng/mL)
1	Standard #1 as prepared above		40
2	120 µL Standard #1	120	20
3	120 µL Standard #2	120	10
4	120 µL Standard #3	120	5
5	120 µL Standard #4	120	2.5
6	120 µL Standard #5	120	1.25
7	120 µL Standard #6	120	0.625
8 (Blank)	N/A	120	0

Sample Preparation

Cell Culture Supernatants: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris. If necessary dilute the samples into Diluent N; user should determine optimal dilution factor depending on dilution needs. Collect supernatants and assay. Undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Milk is recommended for use at 1:2,000 into 1X Diluent N and assay or within the range of 200X – 10000X; 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.

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:100 into 1X Diluent N and assay or within the range of 25X – 800X; 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.

Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1X or within the range of 2X – 10X into Diluent N. 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.

Cell lysate: Rinse cell with cold phosphate-buffered saline (PBS) and then scrape the cell into a tube with 5ml of cold PBS and 1-5 mM EDTA. The concentration for EDTA should be adjusted appropriately depending on the cell type. Centrifuge suspension at 1500rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold lysis buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 10^6 cells, add approximately 100µl of ice-cold lysis buffer. Incubate on ice for 60 minutes. Centrifuge at 13,000 rpm for 30 minutes at 4°C and collect supernatant. If necessary, dilute samples into Diluent N; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Cerebrospinal Fluid (CSF): Collect cerebrospinal fluid (CSF) using sample tube. Centrifuge samples at 3,000 x g for 10 minutes. Dilute samples 1:4000 into 1X Diluent N or within the range of 20x – 20000x; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -80°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Tissue: Extract tissue samples with PBS containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. If necessary, dilute samples into Diluent N; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

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*

Assay Procedure

- 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.
- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, standard solutions and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Complement C3 Standard or sample per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
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 Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µL of 1X Biotinylated Complement C3 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for one hour.
6. Wash microplate as described above.
7. 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.
8. Wash microplate as described above.
9. Add 50 µL of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for about 20 minutes or till the optimal blue color density develops.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
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.
12. Analyze the data as described below.
13. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
14. 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.
15. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Typical Data

Typical standard curve – data provided for demonstration purposes only

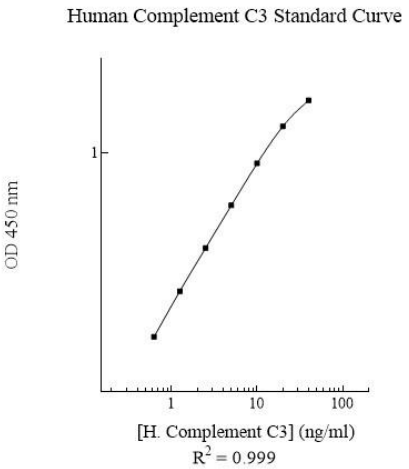

Figure 1. Example of Complement C3 standard curve. The standard curve was prepared as described in Standard preparation section. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Typical Sample Values

SENSITIVITY –
The minimum detectable dose (MDD) of Complement C3 is typically 84 pg/ml.

PRECISION –

	Intra-assay Precision	Inter-Assay Precision
CV (%)	6.0	10.7

SPIKING RECOVERY –
Recovery was determined by spiking one milk and one saliva sample with different complement C3 concentrations.

Sample	Unspiked Sample (ng/ml)	Spiking Value (ng/ml)	Recovery (%)
Milk	3.324	8.054	109
		2.086	108
		0.668	101
Saliva	6.798	8.054	108
		2.086	106
		0.668	90
Average Recovery (%)			104

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.
Milk samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)	
Dilution Factor	Milk
1:1000	93
1:2000	101
1:4000	105

Assay Specificity

This kit recognizes Complement C3 in urine, milk, saliva, cerebrospinal fluid, tissue and cell culture supernatants.

Species Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Equine	None
Monkey	80
Mouse	None
Rat	None
Swine	None
Rabbit	None
Human	100
Protein	**Cross Reactivity (%)**
Human Complement C3b	100%

- No significant cross-reactivity observed with human complement C1, C2, C3a, C3c, C3d, C4, C5, C6, C7, C8, C8G, C9, factor B, factor D, factor H, factor I, and factor P proteins.
- 10% FBS in culture media will not affect the assay.

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)
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. plasma vs. cell extract)	Detection may be reduced or absent in untested sample types

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)

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