Human Complement C3 ELISA Kit

Vertrieb:
L O X O GmbH Immunbiologie Biochemie, Produkte und Systeme
Postfach 11 30 69215 Dossenheim
Telefon +49 (0) 62 21 - 86 80 23 **FAX** +49 (0) 62 21 - 86 80 255
E-Mail: info@loxo.de **Internet:** www.loxo.de

Assaypro LLC
30 Triad South Drive
St. Charles, MO 63304
T (636) 447-9175
F (636) 447-9475
www.assaypro.com

Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank You for choosing Assaypro

Assay Summary

Step 1. Add 25 µl of Standard or Sample and 25 µl of Biotinylated Protein per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 3. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 20 minutes.

Step 4. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax Human Complement C3 ELISA Kit

Catalog No. EC2101-1
Sample Insert/Reference Only

Introduction

Complement component 3 (C3) plays a central role in all three complement activation pathways. The C3 precursor contains 1663 amino acids and has a molecular weight of about 180 kDa (1). Human C3 has 77% identity to mouse C3 at the amino acid level (2). 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 (3). The C3b in complex with receptor can bind covalently to pathogen surfaces to promote phagocytosis (4, 5). Acquired C3 deficiency is associated with severe recurrent meningococcal and pneumococcal infections (6). Plasma C3 and C3a levels are elevated in cryptogenic and large-vessel disease subtypes of ischemic stroke (7).

Principle of the Assay

The AssayMax Human Complement C3 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human complement C3 in **plasma and serum samples**. This assay employs a quantitative **competitive enzyme immunoassay** technique that measures human complement C3 in less than 3 hours. A polyclonal antibody specific for human complement C3 has been pre-coated onto a 96-well microplate with removable strips. Complement C3 in standards and samples is competed with a biotinylated complement C3 sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated protein, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial before opening and using contents.
- The Stop Solution is an acidic solution.

- This kit is for research use only.
- The kit should not be used beyond the expiration date.

Reagents

- **Human Complement C3 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human complement C3.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Complement C3 Standard:** Human complement C3 in a buffered protein base (60 μg, lyophilized).
- **Biotinylated Human Complement C3:** 1 vial, lyophilized.
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP conjugate):** A 100-fold concentrate (80 μl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- 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.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard and Biotinylated Protein at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μl, 20-200 μl, 200-1000 μl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **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. Dilute samples 1:800 into EIA Diluent 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).
- **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. Dilute samples 1:800 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Refer to Sample Dilution Guidelines below for further instruction.

Guidelines for Dilutions of 1:100 or Greater (*for reference only; please follow the protocol for specific dilution suggested)*	
1:100	**1:10000**
A) 4 ul sample: 396 µl buffer = 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.*
1:1000	**1:100000**
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 B : 216 µl buffer (10x) = 100000 fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 60 µg of Human Complement C3 Standard with 2 ml of EIA Diluent to generate a 30 µg/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (30 µg/ml) 1:2 with EIA Diluent

to produce 15, 7.5, 3.75, 1.875, 0.938, and 0.469 μg/ml solutions. EIA Diluent serves as the zero standard (0 μg/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[C3] (μg/ml)
P1	Standard (30 μg/ml)	30.00
P2	1 part P1 + 1 part EIA Diluent	15.00
P3	1 part P2 + 1 part EIA Diluent	7.500
P4	1 part P3 + 1 part EIA Diluent	3.750
P5	1 part P4 + 1 part EIA Diluent	1.875
P6	1 part P5 + 1 part EIA Diluent	0.938
P7	1 part P6 + 1 part EIA Diluent	0.469
P8	EIA Diluent	0.000

- **Biotinylated Human Complement C3 (1x):** Reconstitute Biotinylated Complement C3 with 4 ml EIA Diluent to produce a working solution. Allow the biotin 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.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- 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.
- Add 25 μl of Human Complement C3 Standard or sample per well, and immediately add 25 μl of Biotinylated Human Complement C3 to each well (on top of the standard or sample) and tap plate to mix gently. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- 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.

- Add 50 μl of Streptavidin-Peroxidase conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for 20 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.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- 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.

Data Analysis

- Calculate the mean value of the duplicate or 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 four-parameter or log-log logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

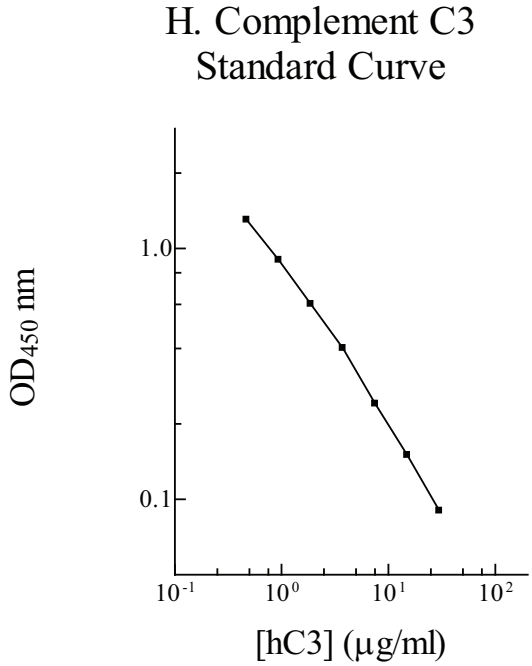
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	µg/ml	OD	Average OD
P1	30.00	0.142 0.133	0.137
P2	15.00	0.183 0.185	0.184
P3	7.500	0.249 0.239	0.244
P4	3.750	0.364 0.368	0.366
P5	1.875	0.516 0.534	0.525
P6	0.938	0.724 0.698	0.711
P7	0.469	1.032 1.034	1.033
P8	0.000	1.912 1.924	1.918
Sample: Human Pool Normal, Sodium Citrate Plasma (800x)		0.591 0.623	0.607

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Reference Value

- The normal human plasma levels of C3 are 500 – 1900 µg/ml.
- Human plasma and serum samples from healthy adults were tested (n=40). On average, C3 level was 1021 µg/ml.

Sample	n	Average Value (µg/ml)
Human Pool Normal Plasma	10	896
Human Normal Plasma	20	1018
Human Pool Normal Serum	10	1149

Performance Characteristics

- The minimum detectable dose of C3 as calculated by 2SD from the mean of a zero standard was established to be 0.2 µg/ml.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	3.0%	2.9%	3.3%	8.8%	9.1%	7.9%
Average CV (%)	3.0%			8.6%		

Spiking Recovery

- Recovery was determined by spiking two plasma samples with different C3 concentrations.

Sample	Unspiked Sample (µg/ml)	Spike (µg/ml)	Expected	Observed	Recovery (%)
1	1.5	1.0	2.5	2.7	108%
		2.5	4.0	4.1	102%
		5.0	6.5	6.6	101%
2	3.0	1.0	4.0	3.7	92%
		2.5	5.5	5.1	93%
		5.0	8.0	8.1	101%
Average Recovery (%)					99%

Linearity

- Plasma and serum samples were serially-diluted to test for linearity.

	Average Percentage of Expected Value (%)	
Sample Dilution	Plasma	Serum
1:400	106%	104%
1:800	99%	98%
1:1600	98%	97%

Cross-Reactivity

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

- No significant cross reactivity observed with Complements C1, C2, C4, C5, C6, C7, C8, and C9.

Troubleshooting

Issue	Causes	Course of Action
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. • Competitive ELISA: If samples generate OD values lower 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.

References

(1) Morris KM et al. (1982) *Science* 215:399-400
(2) de Bruijn MH and Fey GH (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82(3):708-712
(3) Hugli TE (1975) *J Biol Chem.* 250(21):8293-8301
(4) Wiesmann C *et al.* (2006) *Nature*. 444(7116):217-220
(5) Janssen BJ *et al.* (2006) *Nature*. 444(7116):213-216
(6) Ram S *et al.* (2010) *Clin Microbiol Rev.* 23(4):740-780
(7) Stokowska A *et al.* (2011) *Cerebrovasc Dis.* 32(2):114-122

Version 8.0

Related products

- EC1102-1 Human Complement C1r ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC1101-1 Human Complement C1q ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC1111-1 Human Complement C1 ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC2001-1 Human Complement C2 ELISA Kit (Plasma, Serum, Saliva, and Cell Culture samples)
- EC3201-1 Human Complement C3 ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)
- EC3301-1 AssayMax Human Complement C3b ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, CSF, and Cell Culture samples)
- EC2102-1 Human Complement C4 ELISA Kit (Plasma and Serum samples)
- EC3202-1 Human Complement C4 ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)
- EC2202-1 AssayMax Human Complement C4BP ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, CSF, and Cell Culture samples)
- EC5101-1 Human Complement C5 ELISA Kit (Plasma, Serum, Milk, Saliva, and Cell Culture samples)
- EC6101-1 Human Complement C6 ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, and Cell Culture samples)
- EC7101-1 Human Complement C7 ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC8101-1 Human Complement C8 ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC9101-1 Human Complement C9 ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)