Human Complement C8 ELISA Kit

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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

Add 50 µl of standard/samples per well.
Incubate 2 hours.

Wash, then add 50 µl of biotinylated antibody per well.
Incubate 1 hour.

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

Wash, then add 50 µl of TMB per well.
Incubate 15 minutes.

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

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax Human Complement C8 ELISA Kit

Catalog No. EC8101-1
Sample Insert/Reference Only

Introduction

Complement Component 8 (C8) is a 150-kDa complex composed of three genetically distinct subunits: C8α (64 kDa), C8β (64 kDa), and C8γ (22 kDa). C8α and C8β are highly homologous to each other and to C6, C7 and C9, and contain a common membrane attack complex/perforin (MACPF) domain. C8γ has a lipocalin fold and shares no homology with any other complement protein (1). C8 plays a central role in membrane attack complex MAC assembly by coordinating the interaction with complement proteins C5b-7 and the pore-forming protein C9 on pathogen membranes. It is also the first component to penetrate the lipid bilayer (2-3). C8 deficiency exhibits an increased susceptibility to Neisseria meningitidis infections and recurrent meningococcal disease (4).

Principal of the Assay

The AssayMax Human Complement C8 ELISA kit is designed for detection of C8 in human plasma, serum, saliva, milk, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures C8 in less than 4 hours. A polyclonal antibody specific for C8 has been pre-coated onto a microplate. C8 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for C8, which is recognized by a 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, standards, biotinylated antibody, 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 and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **C8 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against C8.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **C8 Standard:** Human C8 in a buffered protein base (40 ng, lyophilized).
- **Biotinylated C8 Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against C8 (140 µl).
- **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, 2 bottles).
- **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

- Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP Conjugate and biotinylated antibody 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 1 month in a vacuum desiccator.

- Diluent (1x) may be stored for up to 1 month at 2-8°C.
- Store standard 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 pipettes).
- 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 1:10000 into EIA Diluent. 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. Remove serum and dilute samples 1:10000 into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Centrifuge samples at 800 x *g* for 10 minutes and assay. Dilute samples 1:20 into EIA Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

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 EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.

- **Standard Curve:** Reconstitute the 40 ng of human C8 standard with 1 ml of EIA Diluent to generate a stock standard solution of 40 ng/ml. 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 C8 standard solution 1:2 with equal volume of EIA Diluent to produce 20, 10, 5, 2.5, 1.25, and 0.625 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within the next 30 days.

Standard Point	Dilution	[C8] (ng/ml)
P1	Standard Solution (40 ng/ml)	40.00
P2	1 part P1 + 1 part EIA Diluent	20.00
P3	1 part P2 + 1 part EIA Diluent	10.00
P4	1 part P3 + 1 part EIA Diluent	5.000
P5	1 part P4 + 1 part EIA Diluent	2.500
P6	1 part P5 + 1 part EIA Diluent	1.250
P7	1 part P6 + 1 part EIA Diluent	0.625
P8	EIA Diluent	0.000

- **Biotinylated C8 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the wash buffer 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, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°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 50 µl of standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.

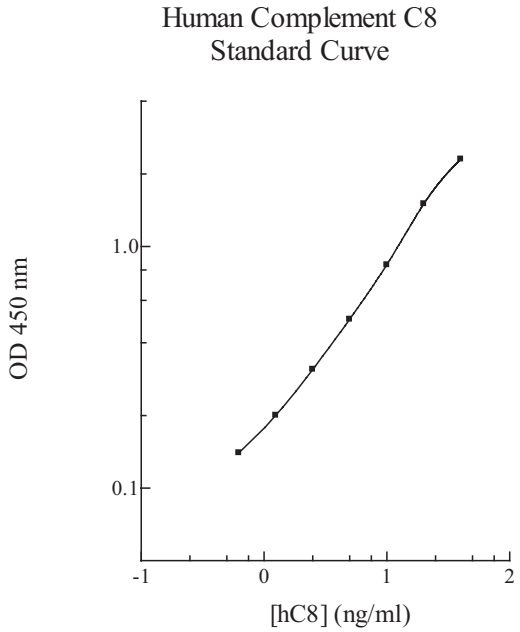
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine, wash six times with 300 μl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 μl of C8 Biotin to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μl of Streptavidin-Peroxidase Conjugate per 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 approximately 15 minutes or till the optimal blue color density develops. Gently tap the 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 high 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 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.

Standard Curve

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



Performance Characteristics

- The minimum detectable dose of C8 is typically ~ 0.6 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7% and 7.3% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:5000	96%	94%
1:10000	100%	99%
1:20000	106%	107%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
1:10	92%
1:20	98%
1:40	103%

Recovery

Standard Added Value	1.5 - 15 ng/ml
Recovery %	83-110%
Average Recovery %	98 %

Cross-Reactivity

Species	**% Cross Reactivity**
Canine	None
Monkey	<20%
Mouse	None
Rat	1%
Swine	None
Rabbit	None
Bovine	None
Proteins	**% Cross Reactivity**
Complement C1	None
Complement C3	None
Complement C4	None
Complement C5	None
Complement C6	None
Complement C7	1%
Complement C8	100%
Complement C9	1%

References

(1) Bubeck D *et al*. (2011) *J Mol Biol*. 405(2):325-330
(2) Hadders MA *et al.* (2007) *Science* 14:317(5844):1552-1554
(3) Lovelace LL *et al.* (2011) *J Biol Chem.* 286(20):17585-17592
(4) Arnold DF *et al.* (2009) *J Clin Immunol.* 29(5):691-695

Version 1.2

Related products

- EC2001-1 Human Complement C2 ELISA Kit (Plasma, Serum, Saliva, and Cell Culture Samples)
- EC2101-1 Human Complement C3 ELISA Kit (Plasma and Serum Samples)
- EC3201-1 Human Complement C3 ELISA Kit (Urine, Milk, Saliva, 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)
- 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, Milk, Saliva, and Cell Culture Samples)
- EC7101-1 Human Complement C7 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)