Human Complement C4 ELISA Kit

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

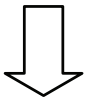
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

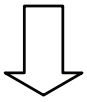
Thank you for choosing Assaypro.

Assay Summary

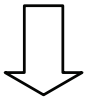
Add 25 µl of Standard/Sample
and 25 µl of Biotinylated Protein per well.
Incubate 2 hours.



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



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 10 minutes.



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

Assay Template

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

AssayMax Human Complement C4 ELISA Kit

Catalog No. EC2102-1
Sample Insert/Reference Only

Introduction

Complement component 4 (C4) plays a key role in the activation of the classical complement pathway. C4 is synthesized as a single-chain precursor molecule (200 kDa) but processed to the three-chain disulphide-linked structure with alpha (93 kDa), beta (78 kDa), and gamma (33 kDa) chains prior to secretion (1, 2). After activation by C1s, C4 is processed to C4a and C4b. C4a anaphylatoxin is a mediator of local inflammation and induces smooth muscle contraction (3). C4b, the major activation product, is an essential subunit of the C3 and C5 convertases of the classical complement pathway. C4 deficiency is associated with systemic lupus erythematosus (5). The C4b degradation product C4d is a marker for humoral rejection in allografts (6).

Principle of the Assay

The AssayMax Human Complement C4 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human complement C4 in plasma and serum. This assay employs a quantitative competitive enzyme immunoassay technique that measures human complement C4 in less than 3 hours. A polyclonal antibody specific for human complement C4 has been pre-coated onto a 96-well microplate with removable strips. Complement C4 in standards and samples is competed by a biotinylated complement C4 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, standards, 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.**
- This kit is for research use only.

- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human Complement C4 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human complement C4.
- **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 C4 Standard:** Human complement C4 in a buffered protein base (20 μg, lyophilized).
- **Biotinylated Human Complement C4:** 1 vial, lyophilized.
- **MIX 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

- Store components of the kit at 2-8°C or -20°C upon arrival 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 1:200 into MIX Diluent or within the range of 1:100 – 1:1000. 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 1:200 into MIX Diluent or within the range of 1:100 – 1:1000. The undiluted samples can be stored 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.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 20 μg of Human Complement C4 Standard with 2 ml of MIX Diluent to generate a stock solution of 10 μg/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 stock solution (10 μg/ml) 1:2 with MIX Diluent to produce 5, 2.5, 1.25, 0.625, and 0.313 μg/ml solutions. MIX 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	[C4] (μg/ml)
P1	1 part Standard (10 μg/ml) + 1 part MIX Diluent	5.000
P2	1 part P1 + 1 part MIX Diluent	2.500
P3	1 part P2 + 1 part MIX Diluent	1.250
P4	1 part P3 + 1 part MIX Diluent	0.625
P5	1 part P4 + 1 part MIX Diluent	0.313
P6	MIX Diluent	0.000

- **Biotinylated Human Complement C4 (4x):** Reconstitute the Biotinylated Human Complement C4 with 4 ml MIX Diluent to produce a 4-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:4 with

MIX Diluent. 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 MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

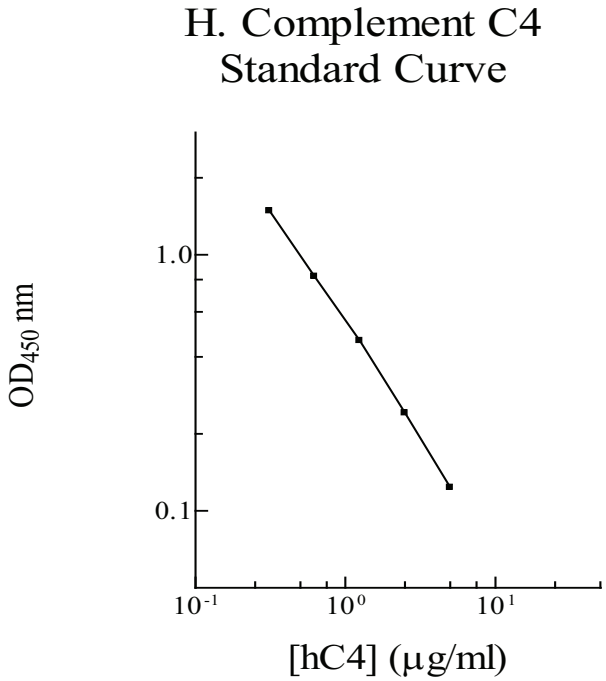
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of Human Complement C4 Standard or sample per well, and immediately add 25 µl of Biotinylated Human Complement C4 to each well (on top of the standard or sample) and 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 about 10 minutes or till 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 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 complement C4 is typically ~ 0.3 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9 % and 7.3 % respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:200	102%	106%
1:400	99%	100%
1:800	93%	92%

Recovery

Standard Added Value	0.4 – 4 ug/ml
Recovery %	88 - 115%
Average Recovery %	99%

Reference Value

- Average normal human plasma complement C4 value is 0.2 – 0.5 mg/ml.

Cross-Reactivity

- No significant cross-reactivity or interference was observed.

References

(1) Roos MH *et al.* (1982) *Nature* 298(5877):854-856
(2) Miura N *et al.* (1987) *J Biol. Chem.* 262(15):7298-7305.
(3) Belt KT *et al.* (1984) *Cell* 36(4):907-914
(4) Moon KE *et al.* (1981) *J Biol Chem.* 256(16):8685-8692
(5) Yang Y *et al.* (2007) *Am J Hum Genet.* 80(6):1037-1054
(6) Girnita AL *et al.* (2008) *Transplantation* 86(2):342-347

Version 5.1

Related products

- EC1111-1 AssayMax Human Complement C1 ELISA Kit (Plasma, Serum, Urine, Milk, Saliva, and Cell Culture samples)
- EC1101-1 AssayMax Human Complement C1q ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, and Cell Culture samples)
- EC1102-1 AssayMax Human Complement C1r ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, and Cell Culture samples)
- EC2001-1 AssayMax Human Complement C2 ELISA Kit (Plasma, Serum, Saliva, and Cell Culture samples)
- EC2101-1 AssayMax Human Complement C3 ELISA Kit (Plasma and Serum samples)
- EC3201-1 AssayMax Human Complement C3 ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)
- EC3202-1 AssayMax Human Complement C4 ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)
- EC5101-1 AssayMax Human Complement C5 ELISA Kit (Plasma, Serum, Saliva, Milk, and Cell Culture samples)
- EC6101-1 AssayMax Human Complement C6 ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, and Cell Culture samples)
- EC7101-1 AssayMax Human Complement C7 ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, and Cell Culture samples)
- EC8101-1 AssayMax Human Complement C8 ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, and Cell Culture samples)
- EC9101-1 AssayMaz Human Complement C9 ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, and Cell Culture samples)