



Human Complement C2 ELISA Kit

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Hinweis/Note:

Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

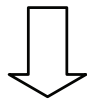
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

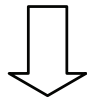
Thank you for choosing Assaypro.

Assay Summary

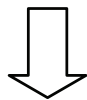
Add 50 μ l of standard/sample per well.
Incubate 2 hours.



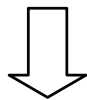
Wash, then add 50 μ l of
biotinylated antibody per well.
Incubate 1 hour.



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



Wash, then add 50 μ l of
Chromogen Substrate per well.
Incubate 15 minutes.



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

AssayMax Human Complement C2 ELISA Kit

Catalog No. EC2001-1
Sample Insert/Reference Only

Introduction

Complement component C2 (C2) is a multi-domain serum protease that has 732 amino acids with 100 kDa. It provides catalytic activity for the C3 and C5 convertases of the complement pathways and plays an important host defense role against microbial infection. Activated complement C1 cleaves C2 into C2a and C2b fragments. The C-terminal 70 kDa fragment C2a consisting of a serine protease (SP) and a von Willebrand factor type A (vWFA) domain. The smaller 30 kDa N-terminal fragment C2b contains 3 complement control protein (CCP) modules (1-3). Polymorphism in C2 is associated with progression to advanced age-related macular degeneration with visual loss (4). Deficiency of C2 is linked with recurrent serious infections and systemic lupus erythematosus (5, 6).

Principle of the Assay

The AssayMax Human Complement C2 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of C2 in plasma, serum, milk, saliva, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures C2 in less than 4 hours. A polyclonal antibody specific for C2 has been pre-coated onto a 96-well microplate with removable strips. C2 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for C2, 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 acidic solution.

Reagents

- **Human C2 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human C2.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human C2 Standard:** Human C2 in a buffered protein base (0.8 µg, lyophilized).
- **Biotinylated Human C2 Antibody (50x):** A 50-fold biotinylated polyclonal antibody against human C2 (140 µl).
- **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, 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 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days 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 μ l, 20-200 μ l, 200-1000 μ l 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. Plasma dilution is suggested at 1:400 into MIX Diluent; 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 (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. Serum dilution is suggested at 1:400 into MIX Diluent; 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 Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples 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:** Collect milk 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.

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 0.8 μ g of Human C2 Standard with 1 ml of MIX Diluent to generate a solution of 0.8 μ g/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 standard solution (0.8 μ g/ml) 1:2 with MIX Diluent to produce 0.4, 0.2, 0.1, 0.05, 0.025, and 0.013 μ g/ml solutions. MIX Diluent serves as the zero standard (0 μ g/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[C2] ($\mu\text{g/ml}$)
P1	Standard (0.8 $\mu\text{g/ml}$)	0.800
P2	1 part P1 + 1 part MIX Diluent	0.400
P3	1 part P2 + 1 part MIX Diluent	0.200
P4	1 part P3 + 1 part MIX Diluent	0.100
P5	1 part P4 + 1 part MIX Diluent	0.050
P6	1 part P5 + 1 part MIX Diluent	0.025
P7	1 part P6 + 1 part MIX Diluent	0.013
P8	MIX Diluent	0.000

- **Biotinylated Human C2 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX 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 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 ($20-25^{\circ}\text{C}$).
- 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 Human C2 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 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 Biotinylated Human C2 Antibody 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 μ l of Chromogen Substrate per well and incubate for about 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 μ l 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.

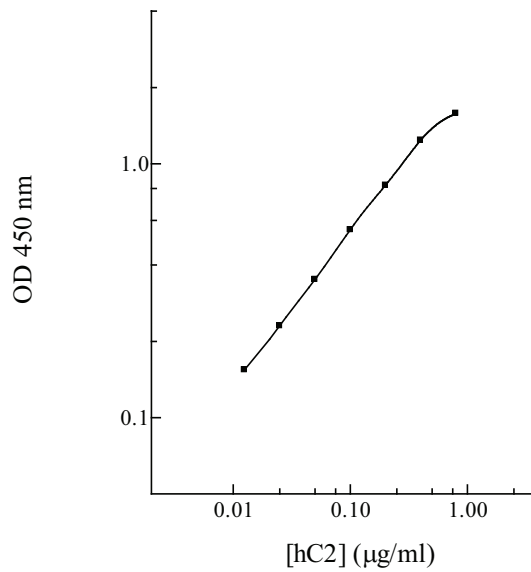
Data Analysis

- 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 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.

Standard Curve

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

Human Complement C2
Standard Curve



Sensitivity and Specificity

- The minimum detectable dose of C2 is typically ~ 0.01 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.5% and 7.4% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:200	96%	95%
1:400	98%	99%
1:800	105%	104%

Recovery

Standard Added Value	0.02 – 0.2 µg/ml
Recovery %	85 - 115%
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Monkey	<30%
Mouse	None
Rat	None
Swine	None
Canine	None
Bovine	None
Rabbit	None
Human	100%
Proteins	% Cross Reactivity
Complement C1	None
Complement C2	100%
Complement C3	None
Complement C4	None
Complement C5	None
Complement C6	None
Complement C7	None
Complement C8	None
Complement C9	None
Complement Factor H	None
Complement Factor B	None

Reference Value

- Normal human C2 plasma levels range from 15 to 50 µg/ml.

References

- (1) Bentley DR (1986) *Biochem. J.* 239:339–345
- (2) Horiuchi T et al. (1989) *J. Immunol.* 142, 2105–2111
- (3) Krishnan V et al. (2009) *Acta Crystallogr D Biol Crystallogr.* 65(Pt 3):266-274
- (4) Francis PJ et al. (2009) *J Med Genet.* 46(5):300-307
- (5) Hellerud BC et al. (2010) *Infect Immun.* 78(2):802-9.
- (6) Aggarwal R et al. (2010) *Lupus.* 19(1): 52–57

Version 1.3

Related Products

- EC1111-1 Human Complement C1 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)
- EC1102-1 Human Complement C1r ELISA Kit (Plasma, Serum, Urine, 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 Sample)
- 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)
- 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)