Human Complement C1r 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.

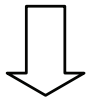
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

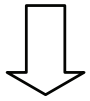
Thank you for choosing Assaypro.

Assay Summary

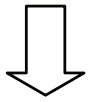
Add 50 µl of Standard/ Sample per well.
Incubate 2 hours.



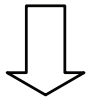
Wash, then add 50 µl of
Biotinylated Antibody per well.
Incubate 1 hour.



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



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 7 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 C1r ELISA Kit

Catalog No. EC1102-1
Sample Insert/Reference Only

Introduction

Complement component C1r is a zymogen of a serine protease that combines with C1q and C1s to form C1, the first component of the classical complement pathway. C1r is a dimer of identical chains and a key mediator of innate immunity. Each precursor contains a 17-amino acid leader peptide, followed by a mature 688-amino acid protein (1). Upon C1q binding to the surface of pathogens, the activated C1r is cleavage into two chains, A and B, connected by disulfide bonds. The non-catalytic amino-terminal C1r A chain (heavy) has 446 amino acids residues (Mr 51 kDa) and contains a growth factor domain and two internal repeats. The catalytic C1r B chain (light) contains 242 amino acids (Mr 27 kDa) and is homologous to the trypsin family of serine proteases. The activated C1r is able to activate C1s which in turn activates C2 and C4, leading to the formation of the membrane attack complex and the elimination of the target (2, 3). C1r deficiency is associated with autoimmune diseases such as systemic lupus erythematosus (4).

Principle of the Assay

The AssayMax Human Complement C1r ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of C1R in human plasma, serum, saliva, urine, milk, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures C1r in less than 4 hours. A polyclonal antibody specific for C1r has been pre-coated onto a 96-well microplate with removable strips. C1r in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for C1r, 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 Complement C1r Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human C1r.
- **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 C1r Standard:** Human C1r in a buffered protein base (36 ng, lyophilized).
- **Biotinylated Human Complement C1r Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human C1r (80 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

- Upon arrival, immediately store components of the kit at recommended temperatures 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 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 1:40000 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. Dilute samples 1:40000 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.
- **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.
- **Urine:** Collect urine 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. Dilute samples 1:2000 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.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2 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.
- **CSF:** Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:40 into EIA Diluent and assay. The undiluted samples can be stored at -80°C 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 the EIA

Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.

- **Standard Curve:** Reconstitute the 36 ng of Human Complement C1r Standard with 4.5 ml of EIA Diluent to generate an 8 ng/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 (8 ng/ml) 1:2 with EIA Diluent to produce 4, 2, 1, 0.5, 0.25, and 0.125 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[C1r] (ng/ml)
P1	Standard (8 ng/ml)	8.000
P2	1 part P1 + 1 part EIA Diluent	4.000
P3	1 part P2 + 1 part EIA Diluent	2.000
P4	1 part P3 + 1 part EIA Diluent	1.000
P5	1 part P4 + 1 part EIA Diluent	0.500
P6	1 part P5 + 1 part EIA Diluent	0.250
P7	1 part P6 + 1 part EIA Diluent	0.125
P8	EIA Diluent	0.000

- **Biotinylated Human Complement C1r Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 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 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 50 µl of Human Complement C1r Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

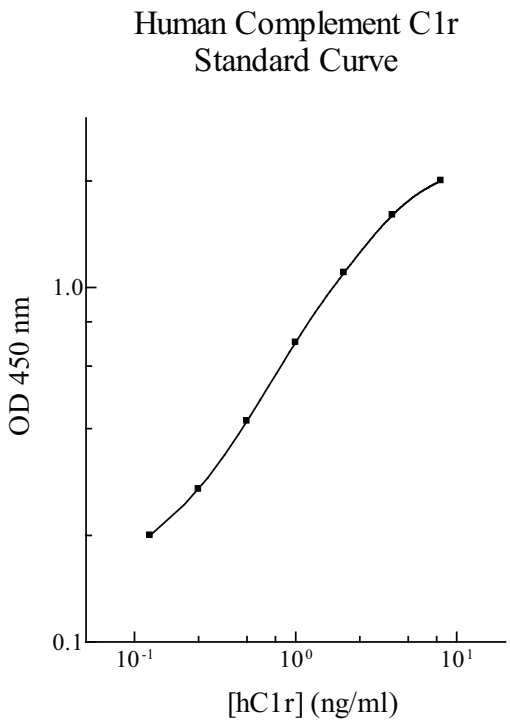
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μl of Biotinylated Human Complement C1r 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 μl of Chromogen Substrate per well and incubate for about 7 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 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.



Sensitivity and Specificity

- The minimum detectable dose of complement C1r is typically ~ 0.1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.1% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:20000	88%	91%
1:40000	99%	99%
1:80000	105%	103%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
1:1000	95%
1:2000	99%
1:4000	104%

	Average Percentage of Expected Value	
Sample Dilution	**Saliva**	**Urine**
No dilution	89%	93%
1:2	99%	94%
1:4	101%	98%

Recovery

Standard Added Value	0.2 – 2.0 ng/ml
Recovery %	84 – 117%
Average Recovery %	98%

Cross-Reactivity

Species	**% Cross Reactivity**
Monkey	None
Mouse	None
Rat	None
Swine	None
Canine	None
Bovine	None
Human	100%
Proteins	**% Cross Reactivity**
Complement C1	100%
Complement C1q	None
Complement C1r	100%
Complement C1s	None
Complement C3	None
Complement C4	None
Complement C5	None
Complement C6	None
Complement C7	None
Complement C8	None
Complement C9	None

Reference Value

- On average, normal human C1r plasma level is 48 µg/ml.

References

(1) Journet A and Tosi M (1986)*Biochem. J*. 240:783-787
(2) Leytus SP *et al.* (1986) *Biochemistry*. 25(17):4855-4863
(3) Arlaud GJ *et al* (1987) *Biochem J*. 241(3):711-720
(4) Wu YL *et al.* (2011) *Lupus.* 20(11):1126-1134

Version 1.2

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)
- 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, CSF, and Cell Culture samples)
- EC3301-1 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, CSF, and Cell Culture samples)
- EC5101-1 Human Complement C5 ELISA Kit (Plasma, Serum, Milk, Saliva, CSF, and Cell Culture samples)
- EC6101-1 Human Complement C6 ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, CSF, 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)