Human Complement Factor I ELISA Kit

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

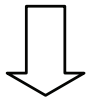
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

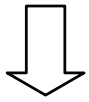
Thank you for choosing Assaypro.

Assay Summary

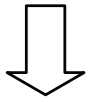
Add 25 µl of standard/sample
and 25 µl of biotinylated protein per well.
Incubate 2 hours.



Wash, then add 50 µl of SP 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 Factor I ELISA Kit

Catalog No. EF8005-1
Sample Insert/Reference Only

Introduction

Complement Factor I (FI), known as C3b/C4b inactivator, is a glycosylated plasma serine proteinase of complement regulatory enzyme. FI is synthesized as a 583-residue single-chain precursor (88 kDa) which is processed into a heterodimer consisting of disulfide-linked heavy (50 kDa) and light (38 kDa) chains (1, 2). It circulates in an inactive zymogen-like state despite being fully processed to the 321-residue mature protein (3). In the presence of additional regulatory cofactors such as C4b-binding protein, factor H, complement receptor 1, and membrane cofactor protein, FI can cleave and inactivate the complement components C3b and C4b to regulate the levels of C3 convertases. FI deficiency is associated with atypical hemolytic uraemic syndrome, primary glomerulonephritis, and recurrent pyogenic infections (4, 5).

Principle of the Assay

The AssayMax Complement Factor I ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of complement factor I in plasma, serum, milk, saliva, and cell culture supernatant. This assay employs a quantitative competitive enzyme immunoassay technique that measures factor I in less than 3 hours. A polyclonal antibody specific for factor I has been pre-coated onto a 96-well microplate with removable strips. Factor I in standards and samples is competed with a biotinylated factor I 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

- **Complement Factor I Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against factor I.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Complement Factor I Standard:** Factor I in a buffered protein base (24 µg, lyophilized).
- **Biotinylated Complement Factor I:** 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, 100x)**: 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 plasma 1:20 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 serum 1:20 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. The samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect human saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute saliva 1:2 into EIA Diluent and assay. The samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect human milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. The 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.
- **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 24 µg of Human Complement Factor I Standard with 1 ml of EIA Diluent to generate a stock solution of 24 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 standard solution (24 µg/ml) 1:2 with EIA Diluent to produce 12, 6, 3, 1.5, 0.75, and 0.375 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	[Factor I] (µg/ml)
P1	Standard (24 µg/ml)	24.00
P2	1 part P1 + 1 part EIA Diluent	12.00
P3	1 part P2 + 1 part EIA Diluent	6.000
P4	1 part P3 + 1 part EIA Diluent	3.000
P5	1 part P4 + 1 part EIA Diluent	1.500
P6	1 part P5 + 1 part EIA Diluent	0.750
P7	1 part P6 + 1 part EIA Diluent	0.375
P8	EIA Diluent	0.000

- **Biotinylated Complement Factor I (1x):** Reconstitute Biotinylated Complement Factor I with 4 ml EIA Diluent to produce a stock 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, 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 25 µl of Complement Factor I Standard and/or sample per well, and immediately add 25 µl of Biotinylated Complement Factor I to each well (on top of the standard or sample). Cover wells with a sealing tape and incubate for 2 hours at room temperature. 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 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.

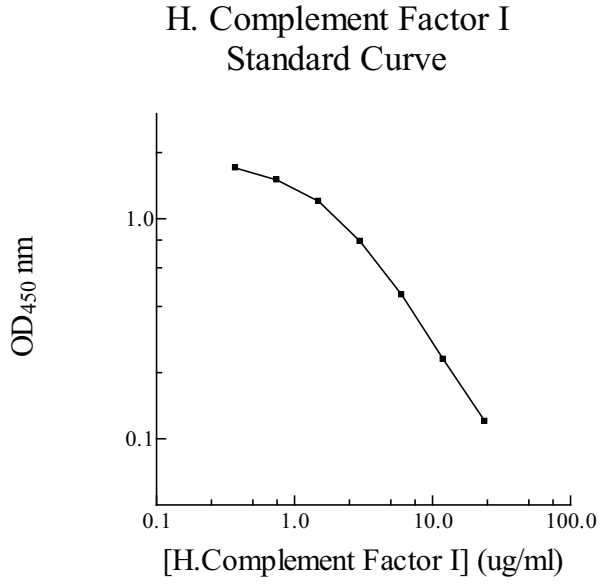
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or until the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to 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 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.



Performance Characteristics

- The minimum detectable dose of FI is typically ~ 0.25 µg/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**
1:10	108%	105%
1:20	98%	99%
1:40	93%	92%

Recovery

Standard Added Value	0.75 - 12 µg/ml
Recovery %	82 - 111%
Average Recovery %	97%

Cross-Reactivity

Species	**% Cross Reactivity**
Canine	<15%
Bovine	None
Monkey	None
Mouse	None
Rat	None
Swine	<15%
Rabbit	None
Proteins	**% Cross Reactivity**
Complement C1	None
Complement C2	None
Complement C3	None
Complement C4	None
Complement C3	None
Complement C4	None
Complement C5	None
Complement C6	None
Complement C7	None
Complement C8	None
Complement C9	None
Complement Factor B	None

Complement Factor D	None
Complement Factor H	None
Complement Factor P	None
Complement Factor I	100%

Reference Value

- On average, normal human factor I plasma level is 40 μg/ml.

References

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(2) Goldberger G *et al.* (1987) *J. Biol. Chem.* 262(21):10065-10071
(3) Roversi P *et al*. (2011) *Proc Natl Acad Sci USA.* 108(31):12839-12844
(4) Bienaime F *et al*. (2010) *Kidney Int.* 77(4):339-249
(5) Sadallah S *et al.* (1999) *Am J Kidney Dis*. 33(6):1153-1157

Version 1.0