Human Complement Factor I

ELISA Kit

Enzyme Immunoassay for the quantification of Human Complement Factor I in Human Serum, plasma, cell culture supernatants, saliva and milk.

Catalog number: ARG82647

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INTRODUCTION

This gene encodes a serine proteinase that is essential for regulating the complement cascade. The encoded preproprotein is cleaved to produce both heavy and light chains, which are linked by disulfide bonds to form a heterodimeric glycoprotein. This heterodimer can cleave and inactivate the complement components C4b and C3b, and it prevents the assembly of the C3 and C5 convertase enzymes. Defects in this gene cause complement factor I deficiency, an autosomal recessive disease associated with a susceptibility to pyogenic infections. Mutations in this gene have been associated with a predisposition to atypical hemolytic uremic syndrome, a disease characterized by acute renal failure, microangiopathic hemolytic anemia and thrombocytopenia. Primary glomerulonephritis with immune deposits and age-related macular degeneration are other conditions associated with mutations of this gene. [provided by RefSeq, Dec 2015]

Trypsin-like serine protease that plays an essential role in regulating the immune response by controlling all complement pathways. Inhibits these pathways by cleaving three peptide bonds in the alpha-chain of C3b and two bonds in the alpha-chain of C4b thereby inactivating these proteins (PubMed:7360115, PubMed:17320177). Essential cofactors for these reactions include factor H and C4BP in the fluid phase and membrane cofactor protein/CD46 and CR1 on cell surfaces (PubMed:2141838, PubMed:9605165, PubMed:12055245). The presence of these cofactors on healthy cells allows degradation of deposited C3b by CFI in order to prevent undesired complement activation, while in apoptotic cells or microbes, the absence of such cofactors leads to C3b-mediated complement activation and subsequent opsonization (PubMed:28671664). [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative competitive enzyme immunoassay technique. An antibody specific for Complement Factor I has been pre-coated onto a microtiter plate. Human Complement Factor I containing samples or standards and a biotin-conjugated Human Complement Factor I are given into the wells of the microtiter plate. Biotin labeled Human Complement Factor I and Human Complement Factor I in standard and samples compete for the antibody binding sites coated on the plate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Complement Factor I bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Complement Factor I in the sample is then determined by comparing the O.D of samples to the standard curve.

The Human Complement Factor I in the samples or Human Complement Factor I in the standards compete with the Human Complement Factor I biotin-conjugated Protein with antibody binding site coated on the plate. High Complement Factor I Protein content in a sample or high concentration of standard results in less biotin-conjugated Complement Factor I -antibody complex binding on the plate, resulting in a low signal. Conversely, low Complement Factor I Protein content in a sample or low concentration of standard result in most biotin-conjugated Complement Factor I-antibody complex binding on the plate, producing a higher signal.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the kit as Storage information below. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard	1 X 19.2 µg (lyophilized)	4°C (store at -20°C after reconstitution)
Biotinylated Human Complement Factor I Protein	1 vial (lyophilized)	4°C (store at -20°C after reconstitution)
HRP-Streptavidin concentrate (100X)	1 vial (80 µl)	-20°C
Dilution Buffer concentrate (10X)	30 ml	4°C
Wash Buffer Concentrate (20X)	30 ml	4°C
TMB substrate	8 ml (Ready to use)	4°C
STOP solution	12 ml (Ready to use)	4°C
Plate sealer	3 strips	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store 100X Streptavidin-HRP concentrate at-20°C.
- Store Standard and Biotinylated Human Complement Factor I Protein at 2-8°C before reconstituting with Dilution Buffer and at -20°C after reconstituting
- Store Microplate, 10X Dilution Buffer concentrate, Wash Buffer, Stop Solution, and TMB 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.
- Briefly spin down the standards and solutions before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- If crystals are observed in the 20X Wash buffer or 10X Dilution Buffer, warm to RT and mix gently until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- All reagents should be mixed by gentle inversion or swirling prior to use.

Do not induce foaming.

- Before opening and using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

a) 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 and collect supernatants. Dilute samples 20X into 1X Dilution Buffer and assay (Dilution factor=20). The undiluted samples can be aliquoted and 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).

b) Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and collect serum. Dilute samples 20X into 1X Dilution Buffer and assay (Dilution factor=20). The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

c) Cell Culture Supernatants: Collect cell culture media and centrifuge at 1500 rpm for 10 minutes at 4°C to remove debris. Collect supernatants and assay. The undiluted samples can be aliquoted and stored at-80°C.

Avoid repeated freeze-thaw cycles.

d) Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Collect supernatant and assay immediately. Sample may be assayed directly without dilution, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

e) Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. It is suggested dilute samples **2X** with 1X Dilution Buffer and assay immediately (Dilution factor=2. User should determine optimal dilution factor depending on application needs). The undiluted samples can be aliquoted and stored at -80°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Note:

- Applicable samples may also include other biofluids, and tissue lysates. If necessary, it is recommended to do pre-test to determine the suitable dilution factor.

- Dilution Note (For duplicate):

a) For 20X dilution: add 10 µL of samples into 190 µL of 1X Dilution Buffer, mix well. (For **serum, plasma**)

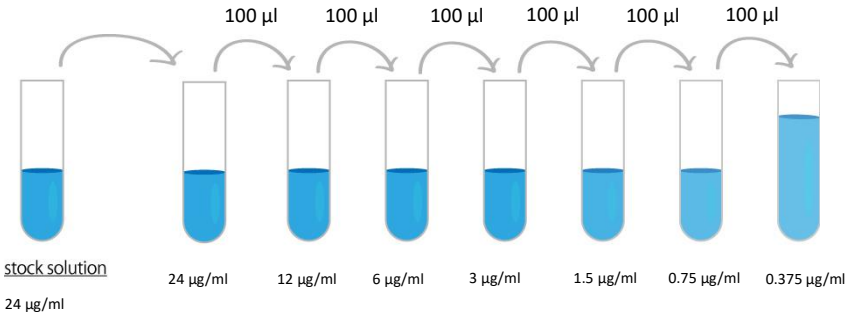
b) For 2X dilution: add 50 µL of samples into 50 µL of 1X Dilution Buffer, mix well. (For **Saliva**)

REAGENT PREPARATION

Freshly dilute all reagents and bring all reagents to room temperature before use.

- 1X Dilution Buffer: Dilute 10X Dilution Buffer concentrate into distilled water to yield 1X Dilution Buffer (E.g. 10 ml of 10X Dilution Buffer + 90 ml of distilled water). If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use. The diluted 1X Diluent can be stored for up to 30 days at 2-8°C.
- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 10 ml of 20X wash buffer + 180 ml of distilled water) If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use.
- Biotinylated Human Complement Factor I Protein: It is recommended to prepare this reagent immediately prior to use. Reconstitute the Biotinylated Human Complement Factor I Protein with 4ml of 1X Dilution Buffer to yield a stock solution. Allow the stock solution to sit for 10 minutes at room temperature (20-25°C) with gentle agitation to make sure the protein is dissolved completely before use. Any remaining solution should be aliquoted and stored at-20°C for up to 30 days. Avoid repeated freeze-thaw cycles
- 1X HRP-Streptavidin Solution: It is recommended to prepare this reagent immediately prior to use. Spin down the 100X HRP-Streptavidin Solution concentrate briefly and dilute the desired amount of the conjugate with 1X Dilution Buffer (E.g. 40 µl of Streptavidin-HRP conjugate + 3960 µl of 1X Dilution Buffer). Any remaining solution should be frozen at-20°C.

- **Sample**: If the initial assay found samples contain Complement Factor I higher than the highest standard, the samples can be diluted with 1X Dilution Buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.
  (It is recommended to do pre-test to determine the suitable dilution factor).
- **Standards**: Reconstitute the standard with **800 µl** of 1X Dilution Buffer to yield a stock concentration of **24 µg/ml**. Allow the stock standard to sit for 10 minutes at room temperature (20-25°C) with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The 1X Dilution Buffer serves as zero standard (0 µg/ml), and the rest of the standard serial dilution can be diluted with 1X Dilution Buffer as according to the suggested concentration below: **24 µg/ml**, **12 µg/ml**, **6 µg/ml**, **3 µg/ml**, **1.5 µg/ml**, **0.75 µg/ml**, **0.375 µg/ml**.
  Note: Any remaining stock solution should be stored at -20°C and used within 2 days. Aliquot to avoid repeated freeze-thaw cycles.



Dilute Complement Factor I standard as according to the table below:

Standard	Complement Factor I Conc. (µg/ml)	µl of Dilution Buffer	µl of standard
S7	24 µg/ml	0	200 (24 µg/ml Stock)
S6	12 µg/ml	100	100 (S7)
S5	6 µg/ml	100	100 (S6)
S4	3 µg/ml	100	100 (S5)
S3	1.5 µg/ml	100	100 (S4)
S2	0.75 µg/ml	100	100 (S3)
S1	0.375 µg/ml	100	100 (S2)
S0	0	100	0

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 (RT, 20-25°C). When diluting samples and reagents, they must be mixed completely and evenly. Standard Complement Factor I detection curve should be prepared for each experiment. Standards, samples and controls should be assayed in duplicates.

1. 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. The remaining microplate strips may be stored for up to 30 days in a vacuum desiccator.
2. Add **25 µl** of **standards, samples and zero controls** (S0, Dilution Buffer) into wells.

3. Immediately add 25 µl of Biotinylated Human Complement Factor I Protein to each well (on the top of the standard or sample).
4. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
5. Cover wells with a sealing tape and incubate for 2 hours at RT (20-25°C). Start the timer after the last addition.
6. Remove sealer from plate.
7. Aspirate each well and wash, repeating the process 4 times for a total 5 washes (If a microplate washer is used, wash the wells for a total 6 washes). Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
8. Add 50 µl of 1X HRP-Streptavidin Solution into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
9. Reseal the plate with sealer. Incubate for 30 minutes at RT. (Turn on the microplate reader and set up the program in advance.)
10. Wash as according to step 7.
11. Add 50 µl of TMB substrate solution into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
12. Incubate for 8 minutes at RT or until the optimal blue color density develops. (Protect from light)
13. Add 50 µl of STOP solution into all wells to stop the reaction. The color of the solution should change from blue to yellow. Gently tap plate to ensure

thorough mixing. Break any bubbles that may have formed.

14. Read the OD with a microplate reader at **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. So it is recommended read the absorbance **within 10 min** after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

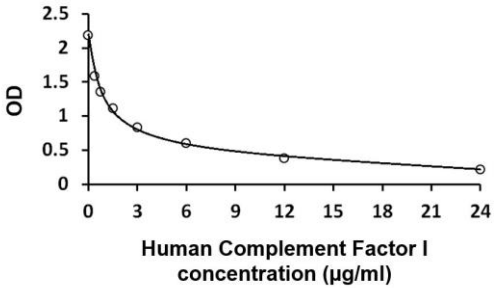
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human Complement Factor I ranged from 0.375- 24 µg/ml. The mean MDD was 0.28 µg/ml.

Specificity

This assay recognizes natural and recombinant Human Complement Factor I.

Cross-Reactivity:

Pig: 75%

Rat: 5%

Mouse, Monkey, Bovine, Dog and Rabbit: None

No significant cross-reactivity observed with complement factor B, factor D, factor H, factor P, C1, C2, C3, C4, C5, C6, C7, C8 and C9.

Recovery

89-111%

Linearity

93-106% for plasma and serum samples.

Intra-assay and Inter-assay precision

The CV values of intra-assay was 5.4% and inter-assay was 9.8%.