Human C5 / Complement 5 ELISA Kit

Enzyme Immunoassay for the quantification of Human C5 / Complement 5 in Human Serum, plasma, cell culture supernatants, saliva, milk and CSF.

Catalog number: ARG82649

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INTRODUCTION

This gene encodes a component of the complement system, a part of the innate immune system that plays an important role in inflammation, host homeostasis, and host defense against pathogens. The encoded preproprotein is proteolytically processed to generate multiple protein products, including the C5 alpha chain, C5 beta chain, C5 anaphylatoxin and C5b. The C5 protein is comprised of the C5 alpha and beta chains, which are linked by a disulfide bridge. Cleavage of the alpha chain by a convertase enzyme results in the formation of the C5 anaphylatoxin, which possesses potent spasmogenic and chemotactic activity, and the C5b macromolecular cleavage product, a subunit of the membrane attack complex (MAC). Mutations in this gene cause complement component 5 deficiency, a disease characterized by recurrent bacterial infections. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Nov 2015]

Activation of C5 by a C5 convertase initiates the spontaneous assembly of the late complement components, C5-C9, into the membrane attack complex. C5b has a transient binding site for C6. The C5b-C6 complex is the foundation upon which the lytic complex is assembled.

Derived from proteolytic degradation of complement C5, C5 anaphylatoxin is a mediator of local inflammatory process. Binding to the receptor C5R1 induces a variety of responses including intracellular calcium release, contraction of smooth muscle, increased vascular permeability, and histamine release from mast cells and basophilic leukocytes (PubMed:8182049). C5 is also a potent chemokine which stimulates the locomotion of polymorphonuclear leukocytes and directs their migration toward sites of inflammation. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for C5 / Complement 5 has been pre-coated onto a microtiter plate. Human C5 / Complement 5 standards or samples are pipetted into the wells and any C5 / Complement 5 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for C5 / Complement 5 is added to each well and incubate. 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 C5 / Complement 5 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 C5 / Complement 5 in the sample is then determined by comparing the O.D of samples to the standard curve.

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 28 ng (lyophilized)	4°C (store at -20°C after reconstitution)
Antibody conjugate concentrate (50X)	1 vial (120 µl)	-20°C
HRP-Streptavidin concentrate (100X)	1 vial (80 µl)	-20°C
Dilution Buffer concentrate (10X)	30 ml	4°C
Wash Buffer Concentrate (20X)	2 X 30 ml	4°C
TMB substrate	7 ml (Ready to use)	4°C
STOP solution	11 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.
- Store the components at recommended temperature.
- Store 50X Antibody conjugate concentrate and 100X Streptavidin-HRP concentrate at-20°C.
- Store Standard at 2-8°C before reconstituting with Dilution Buffer and at -20°C after reconstituting with Dilution Buffer.
- 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 20,000X into 1X Dilution Buffer and assay (Dilution factor=20000). 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 20,000X into 1X Dilution Buffer and assay (Dilution factor=20000). 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. It is suggested dilute samples 40X with 1X Dilution Buffer and

assay immediately (Dilution factor=40. 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) CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. It is suggested dilute samples **20X** with 1X Dilution Buffer and assay immediately (Dilution factor=20. 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.

f) Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. It is suggested dilute samples **8X** with 1X Dilution Buffer and assay immediately (Dilution factor=8. 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.

Note:

- Applicable samples may also include other biofluids, and tissue lysates. If necessary, user should determine optimal dilution factor depending on application needs.

- Dilution Note:

a) For 100X dilution: add 5 µL of samples into 495 µL of 1X Dilution Buffer, mix well.

b) For 20000X dilution: add 5 µL of diluted samples from **a)** into 995 µL of 1X Dilution Buffer, mix well. (For **serum, plasma**)

c) For 20X dilution: add 10 µL of samples into 190 µL of 1X Dilution Buffer, mix well. (For CSF)

d) For 40X dilution: add 10 µL of samples into 390 µL of 1X Dilution Buffer, mix well. (For Milk)

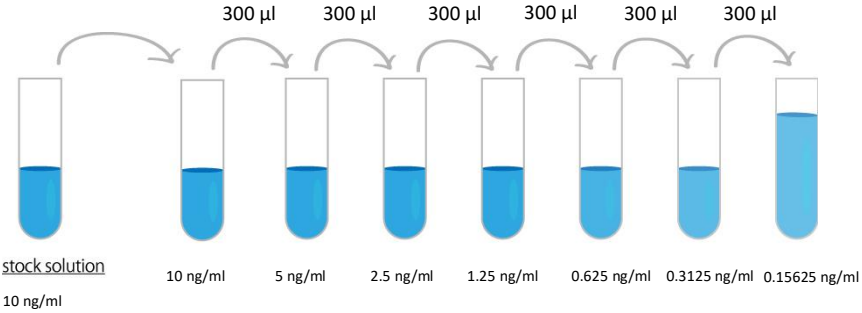
e) For 8X dilution: add 20 µL of samples into 140 µL of 1X Dilution Buffer, mix well. (For Saliva)

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.
- 1X Antibody conjugate: It is recommended to prepare this reagent immediately prior to use. Briefly spin down the 50X antibody conjugate concentrate. Dilute 50X antibody conjugate concentrate into 1X Dilution Buffer to yield 1X detection antibody solution. (e.g. 60 µl of 50X antibody conjugate concentrate + 2940 µl of 1X Dilution Buffer). Any remaining solution should be frozen at-20°C.

- 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 C5 / Complement 5 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 2.8 ml of 1X Dilution Buffer to yield a stock concentration of 10 ng/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 ng/ml), and the rest of the standard serial dilution can be diluted with 1X Dilution Buffer as according to the suggested concentration below: 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.3125 ng/ml, 0.15625 ng/ml.
  Note: Any remaining stock solution should be stored at -20°C and used within 30 days. Aliquot to avoid repeated freeze-thaw cycles is recommended.



Dilute C5 / Complement 5 standard as according to the table below:

Standard	C5 / Complement 5 Conc. (ng/ml)	µl of Dilution Buffer	µl of standard
S7	10 ng/ml	0	500 (10 ng/ml Stock)
S6	5 ng/ml	200	200 (S7)
S5	2.5 ng/ml	200	200 (S6)
S4	1.25 ng/ml	200	200 (S5)
S3	0.625 ng/ml	200	200 (S4)
S2	0.3125 ng/ml	200	200 (S3)
S1	0.15625 ng/ml	200	200 (S2)
S0	0	200	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 C5 / Complement 5 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 **50 µl** of **standards, samples and zero control**s (S0, Dilution Buffer) into wells. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
3. Cover wells with a sealing tape and incubate for **2 hours at RT (20-25°C)**. Start the timer after the last addition.
4. Remove sealer from plate.
5. 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.

6. Add **50 µl** of **1X Antibody conjugate** into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
7. Reseal the plate with sealer. Incubate for **1 hour at RT**.
8. **Wash as** according to **step 5**.
9. 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.
10. Reseal the plate with sealer. Incubate for **30 minutes at RT.** (Turn on the microplate reader and set up the program in advance.)
11. **Wash as** according to **step 5**.
12. 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.
13. Incubate for **15 minutes at RT** or until the optimal blue color density develops. (Protect from light)
14. Add **50 µl** of **STOP solution** into all wells to stop the reaction. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
15. 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.

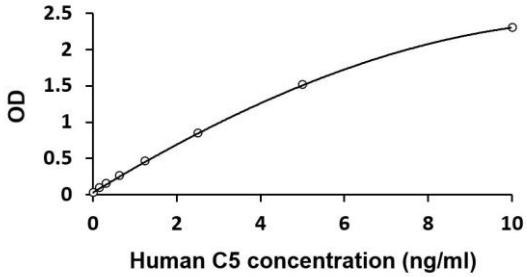
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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 C5 / Complement 5 ranged from 0.156- 10 ng/ml. The mean MDD was 0.084 ng/ml.

Specificity

This assay recognizes native Human C5 / Complement 5. No significant cross-reactivity or interference with the factors below was observed:

Cross-Reactivity:

Monkey: 75%

Mouse, Rat, Pig, Dog, Bovine and Rabbit: None

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

Linearity

Plasma: 97 - 103%

Serum: 94 - 110%

Intra-assay and Inter-assay precision

The CV values of intra-assay was 4.8% and inter-assay was 9.2%.