



AssayMax Human Complement C3 ELISA Kit

Catalog No. EC2101-1

Introduction

Complement component 3 (C3) plays a central role in all three complement activation pathways. The C3 precursor contains 1663 amino acids and has a molecular weight of about 180 kDa (1). Human C3 has 77% identity to mouse C3 at the amino acid level (2). C3 is cleaved by C3 convertase into two activated fragments C3a and C3b. The anaphylatoxin C3a is a vasoactive peptide and a mediator of local inflammatory process (3). The C3b in complex with receptor can bind covalently to pathogen surfaces to promote phagocytosis (4-5). Acquired C3 deficiency is associated with severe recurrent meningococci and pneumococci infections (6). Plasma C3 and C3a levels are elevated in cryptogenic and large-vessel disease subtypes of ischemic stroke (7).

Principal of the Assay

The AssayMax Human Complement C3 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human complement C3 in plasma and serum. This assay employs a quantitative competitive enzyme immunoassay technique that measures human complement C3 in less than 3 hours. A polyclonal antibody specific for human complement C3 has been pre-coated onto a 96-well microplate with removable strips. Complement C3 in standards and samples is competed with a biotinylated complement C3 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 acid solution.

Reagents

- **Human Complement C3 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human complement C3.

- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Complement C3 Standard:** Human Complement C3 in a buffered protein base (60 µg, lyophilized).
- **Biotinylated Complement C3:** 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):** 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 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store SP Conjugate at -20⁰C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8⁰C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8⁰C.
- Store Standard and Biotinylated Protein at 2-8⁰C before reconstituting with Diluent and at -20⁰C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000µl 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 2000 x g for 10 minutes and assay. Dilute samples 1:800 into EIA Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:800 into EIA Diluent. The undiluted samples can be stored at -20⁰C 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 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **Standard Curve:** Reconstitute the 60 µg of Complement C3 Standard with 2 ml of EIA Diluent to generate a solution of 30 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 (30 µg/ml) 1:2 with EIA Diluent to produce 15, 7.5,

3.75, 1.88, 0.94 and 0.47 µg/ml solutions. EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[Complement C3] (µg/ml)
P1	Standard (30 µg/ml)	30.0
P2	1 part P1 + 1 part EIA Diluent	15.0
P3	1 part P2 + 1 part EIA Diluent	7.50
P4	1 part P3 + 1 part EIA Diluent	3.75
P5	1 part P4 + 1 part EIA Diluent	1.88
P6	1 part P5 + 1 part EIA Diluent	0.94
P7	1 part P6 + 1 part EIA Diluent	0.47
P8	EIA Diluent	0.00

- **Biotinylated Complement C3 (2x):** Dilute Biotinylated Complement C3 with 4 ml EIA Diluent to produce a 2-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:2 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

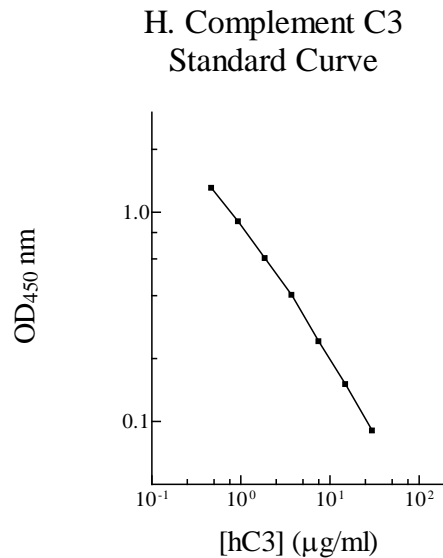
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of standard or sample per well, and immediately add 25 µl of Biotinylated Complement C3 to each well (on top of the Standard or sample) and mix gently. Cover wells with a sealing tape and incubate for two 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 it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel 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 8 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 log-log or four-parameter 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 complement C3 is typically ~ 0.5 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.6 % and 7.1 % respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:400	101%	102%
1:800	98%	101%
1:1600	96%	97%

Recovery

Standard Added Value	0.5 – 15 ug/ml
Recovery %	86 - 105 %
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Canine	2%
Bovine	0.01%
Monkey	5 %
Mouse	0.5%
Rat	1%
Swine	20%
Rabbit	0.05%
Human	100%

References

- (1) Morris KM et al. (1982) *Science* 215:399-400
- (2) de Bruijn MH and Fey GH (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82(3):708-712
- (3) Hugli TE (1975) *J Biol Chem.* 250(21):8293-8301
- (4) Wiesmann C et al. (2006) *Nature.* 444(7116):217-220
- (5) Janssen BJ et al. (2006) *Nature.* 444(7116):213-216
- (6) Ram S et al. (2010) *Clin Microbiol Rev.* 23(4):740-780
- (7) Stokowska A et al. (2011) *Cerebrovasc Dis.* 32(2):114-122

Version 6.9

Related products

- EC2102-1 AssayMax Human Complement C4 ELISA Kit (Plasma and Serum samples)
- EC3201-1 AssayMax Human Complement C3 ELISA Kit (Urine, Saliva, Milk and Cell Culture Supernatant samples)
- EC3202-1 AssayMax Human Complement C4 ELISA Kit (Urine, Saliva, Milk and Cell Culture Supernatant samples)