

Human Complement C7 ELISA Kit

Introduction

Human Complement Component 7 (C7) C7 is a single-chain glycoprotein consisting of 821 amino acid residues with a molecular weight of about 97 kDa. The amino-terminal of C7 has 23-30% homology with Complement Components C8 and C9 (1). C7 is one of five complement proteins C5b, C6, C7, C8, and C9 that assemble on pathogen membranes to form the lethal membrane attack complex. It serves as a membrane anchor for the C5b-7 complex. C7 deficiency exhibits an increased susceptibility to *Neisseria meningitidis* infections and recurrent meningococcal disease (2-3). Plasma C7 is also associated with asthma exacerbation (4), age-related macular degeneration (5), and multiple sclerosis (6).

Principal of the Assay

The Human C7 ELISA kit is designed for detection of C7 in human plasma, serum, urine, milk, saliva, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures C7 in 4 hours. A polyclonal antibody specific for C7 has been pre-coated onto a microplate. C7 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for C7, 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 acid solution.

Reagents

- **Human C7 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human C7.
- **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 C7 Standard:** Human C7 in a buffered protein base (160 ng, lyophilized).

- **Biotinylated C7 Antibody (50x):** A 50-fold biotinylated polyclonal antibody against human C7 (140 µl).
- **MIX 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

- Store components of the kit at 2-8°C or -20°C upon arrival 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
- 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 2-8°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, 200-1000µl 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 1:5000 into MIX Diluent. Store samples at -20°C 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. Dilute samples 1:5000 into MIX Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 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 600 x g for 10 minutes and assay. Urine dilution is suggested at 1:2 in MIX Diluent; however, the user should determine the optimal dilution factor. 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 600 x g for 10 minutes and assay. Dilute samples 1:50 into MIX Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:4 into MIX Diluent. Store samples 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.
- MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- Standard Curve:** Reconstitute the 160 ng of C7 Standard with 4 ml of MIX Diluent to generate a solution of 40 ng/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 (40 ng/ml) 1:2 with MIX Diluent to produce 20, 10, 5, 2.5, 1.25 and 0.625 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[C7] (ng/ml)
P1	Standard Stock (40 ng/ml)	40.00
P2	1 part P1 + 1 part MIX Diluent	20.00
P3	1 part P2 + 1 part MIX Diluent	10.00
P4	1 part P3 + 1 part MIX Diluent	5.000
P5	1 part P4 + 1 part MIX Diluent	2.500
P6	1 part P5 + 1 part MIX Diluent	1.250
P7	1 part P6 + 1 part MIX Diluent	0.625
P8	MIX Diluent	0.000

- Biotinylated C7 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX 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 MIX 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 50 µl of Standard or sample per well. 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 Biotinylated C7 Antibody to each well and incubate for one 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.

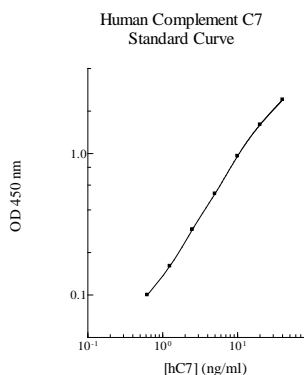
- Add 50 µl of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue 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 C7 is typically 0.6 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.3% and 7.1% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:2500	91%	90%
1:5000	99%	98%
1:10000	102%	103%

Sample Dilution	Average Percentage of Expected Value	
	Milk	
1:25	91%	
1:50	95%	
1:100	93%	

	Average Percentage of Expected Value	
Sample Dilution	Saliva	Urine
1:2	89%	99%
1:4	99%	96%
1:8	105%	104%

Recovery

Standard Added Value	1.0 – 10 ng
Recovery %	81-114%
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Monkey	50% (Suggest 1:2000 dilutions for Plasma/Serum)
Mouse	None
Rat	None
Swine	None
Canine	0.5%
Bovine	None
Rabbit	None

- No significant cross-reactivity observed for C1, C3, C4, C5, C6, C8, C9

References

- (1) DiScipio RG *et al.* (1988) *J Biol Chem.* 263(1):549-560
- (2) Nürnberger W *et al.* (1989) *Eur J Pediatr.* 148(8):758-760
- (3) Kuijpers TW *et al.* (2010) *Mol Immunol.* 47(4):671-677
- (4) Nishioka T *et al.* (2008) *Proteomics Clin Appl.* 2(1):46-54
- (5) Dinu V *et al.* (2007) *Genet Epidemiol.* 31(3):224-237
- (6) Kallio SP *et al.* (2009) *Hum Mol Genet.* 18(9):1670-1683

Version 1.4