Human Complement C4 ELISA Kit

Introduction

Complement protein C4 is the second component to react in the complement sequence. It is a beta-globulin with a sedimentation coefficient of 18.7 and a molecular weight of 240,000. The C4 component participates in the initial step of activation of classical complement pathway (1). Lower levels of complement C4 in serum was associated with primary biliary cirrhosis (2), human systemic lupus erythematosus (3), and chronic liver disease (4).

Principal of the Assay

The Human complement C4 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human complement C4 in plasma and serum. This assay employs a quantitative competitive enzyme immunoassay technique that measures human complement C4 in less than 3 hours. A polyclonal antibody specific for human complement C4 has been pre-coated onto a 96-well microplate with removable strips. Complement C4 in standards and samples is competed by a biotinylated complement C4 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

- 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 C4 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human complement C4.
- **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 C4 Standard:** Human Complement C4 in a buffered protein base (7.5 μg, lyophilized).
- **Biotinylated Complement C4:** 1 vial, lyophilized.
- **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 kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8^{0}C.
- Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

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 MIX Diluent. The undiluted samples can be stored at <-20^{0}C 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 MIX Diluent. The undiluted samples can be stored at <-20^{0}C 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. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 7.5 µg of complement C4 Standard with 1.5 ml of MIX Diluent to generate a stock solution of 5 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 stock solution (5 µg/ml) 1:2 with MIX Diluent to produce 2.5, 1.25, 0.625 and 0.313 µg/ml solutions. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[Complement C4] (µg/ml)
P1	Standard Stock (5 µg/ml)	5.000
P2	1 part P1 + 1 part MIX Diluent	2.500
P3	1 part P2 + 1 part MIX Diluent	1.250
P4	1 part P3 + 1 part MIX Diluent	0.625
P5	1 part P4 + 1 part MIX Diluent	0.313
P6	MIX Diluent	0.000

- **Biotinylated Complement C4 (2x):** Dilute Biotinylated complement C4 with 4 ml MIX 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 MIX Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** 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^{0}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^{0}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 C4 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 a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till 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.

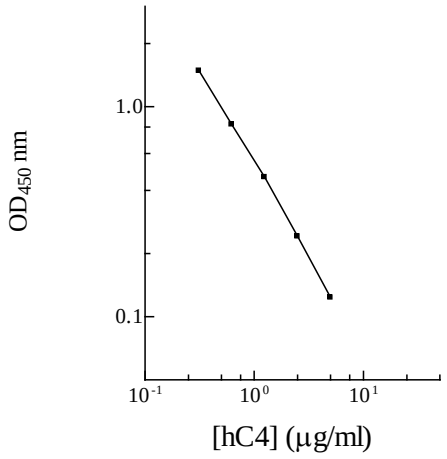
Data Analysis

- 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.

H. Complement C4
Standard Curve



Performance Characteristics

- The minimum detectable dose of complement C4 is typically 300 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.5 % respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:400	99%	101%
1:800	100%	102%
1:1600	96%	98%

Recovery

Standard Added Value	0.1 – 2 ug/ml
Recovery %	88-115 %
Average Recovery %	103 %

Reference Value

Average normal human plasma complement C3 value is 0.43 g/L.

Cross-Reactivity

- No significant cross-reactivity or interference was observed.

References

(1) Samano ES *et al.* (2004) *Rev Hosp Clin Fac Med Sao Paulo.* 59(3): 138-44
(2) Gardinali M *et al.* (1998) *Clin Immunol Immunopathol* 87(3): 297-303
(3) Yang Y *et al.* (2004) *Curr Dir Autoimmun* 7:98-132
(4) Schirren CA *et al.* (1995) *Dig Dis Sci* 40(6): 1221-5

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