Human Complement C1 ELISA Kit

Catalog No: IRAPKT4010 **Lot No**: SAMPLE

INTENDED USE

The Human Complement C1 ELISA kit is designed for detection of C1 in human plasma, serum, saliva, urine, milk, and cell culture samples.

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INTRODUCTION

Complement component C1 (C1) is a calcium-dependent serine protease complex with an approximate mass of 790 kDa and acts as the first component of the classical complement pathway. C1 is formed from the association of a recognition protein C1q and two catalytic subunits C1r and C1s respectively (1 - 2). The globular heads of the C1q bind to the Fc-fragment of IgM or IgG on the surface of a pathogen, resulting in the activation of C1r. The activated C1r is able to activate C1s which in turn activates C2 and C4, leading to the production of the C4b-C2a form of C3-convertase (3 - 4). C1 deficiency is generally associated with severe immune complex disease of systemic lupus erythematosus and glomerulonephritis (5 - 6).

PRINCIPLE OF THE ASSAY

The Human Complement C1 ELISA kit is designed for detection of C1 in human plasma, serum, saliva, urine, milk, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures C1 in less than 4 hours. A polyclonal antibody specific for C1 has been pre-coated onto a microplate. C1 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for C1, 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 acidic solution.

REAGENTS

- **Human Complement C1 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human C1.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Complement C1 Standard:** Human Complement C1 in a buffered protein base (256 ng, lyophilized).
- **Biotinylated Human Complement C1 Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human complement C1 (80 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.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. 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, 200-1000 µl 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 3000 x *g* for 10 minutes. Dilute samples 1:40000 into MIX Diluent and assay. The undiluted samples can be 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.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:40000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 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 800 x *g* for 10 minutes and assay. 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 800 x *g* for 10 minutes. Dilute samples 1:40 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. 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 Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- **Standard Curve:** Reconstitute the 256 ng of Human Complement C1 Standard with 4 ml of MIX Diluent to generate a solution of 64 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution (64 ng/ml) should be further diluted 1:8 with MIX Diluent to produce a 8 ng/ml standard solution. Prepare duplicate or triplicate standard points by serially diluting the standard solution (8 ng/ml) 1:2 with MIX Diluent to produce 4, 2, 1, 0.5, 0.25, and 0.125 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[C1] (ng/ml)
P1	1 part Standard (64 ng/ml) + 7 parts MIX Diluent	8.000
P2	1 part P1 + 1 part MIX Diluent	4.000
P3	1 part P2 + 1 part MIX Diluent	2.000
P4	1 part P3 + 1 part MIX Diluent	1.000
P5	1 part P4 + 1 part MIX Diluent	0.500
P6	1 part P5 + 1 part MIX Diluent	0.250
P7	1 part P6 + 1 part MIX Diluent	0.125
P8	MIX Diluent	0.000

- Biotinylated Human Complement C1 Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 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

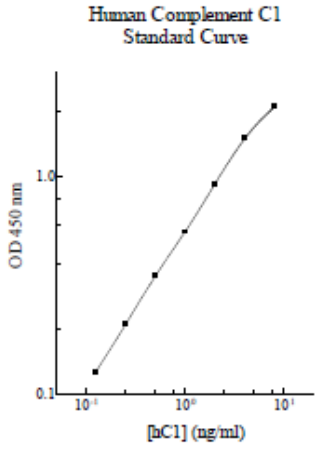
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human Complement C1 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 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 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human Complement C1 Antibody to each well and incubate for 1 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.



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SENSITIVITY AND SPECIFICITY

- The minimum detectable dose of C1 is typically ~ 0.1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7% and 7.0% respectively.

LINEARITY

	Average Percentage of Expected Value	
Sample Dilution	Plasma	Serum
1:20000	88%	93%
1:40000	99%	98%
1:80000	104%	105%

	Average Percentage of Expected Value
Sample Dilution	Milk
1:20	91%
1:40	99%
1:80	105%

	Average Percentage of Expected Value	
Sample Dilution	Saliva	Urine
No dilution	87%	91%
1:2	96%	98%
1:4	106%	105%

RECOVERY

Standard Added Value	0.3 – 5 ng/ml
Recovery %	84-115 %
Average Recovery %	98 %

CROSS-REACTIVITY

Species	% Cross Reactivity
Monkey	<5%
Mouse	None
Rat	None
Swine	None
Canine	<5%
Bovine	None
Human	100%
Proteins	**% Cross Reactivity**
Complement C1	100%
Complement C2	None
Complement C3	None
Complement C4	None
Complement C5	None
Complement C6	None
Complement C7	None
Complement C8	None
Complement C9	None

REFERENCE VALUE

- On average, normal human complement C1 plasma level is 75 µg/ml.

REFERENCES

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(6) Mii A *et al.* (2009) *Clin Exp Nephrol.* 13(4):263-274

Assay Template

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

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