Human Complement C3b ELISA Kit

Catalog No: IRAPKT4013 **Lot No**: SAMPLE

INTENDED USE

The Human Complement C3b ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human complement C3b in plasma, serum, urine, milk, saliva, CSF, and cell culture supernatant samples.

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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 meningococcal and pneumococcal infections (6). Plasma C3 and C3a levels are elevated in cryptogenic and large-vessel disease subtypes of ischemic stroke (7).

PRINCIPLE OF THE ASSAY

The Human Complement C3b ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human complement C3b in plasma, serum, urine, milk, saliva, CSF, and cell culture supernatant samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human complement C3b in less than 4 hours. A polyclonal antibody specific for human complement C3b has been pre-coated onto a 96-well microplate with removable strips. Complement C3b in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for complement C3b, 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 C3b Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human complement C3b.
- **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 C3b Standard:** Human complement C3b in a buffered protein base (20 ng, lyophilized).
- **Biotinylated Complement C3b Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against complement C3b (140 µl).
- **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, 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 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days 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 1:400000 into EIA 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 and remove serum. Dilute samples 1:400000 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:4 into EIA Diluent and assay. If necessary, dilute samples within the range of 1:2 to 1:8. 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 pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:100 into EIA 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. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Milk dilution is suggested at 1:4000 into EIA Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **CSF**: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:1000 into EIA Diluent and assay. The undiluted samples can be stored at -80°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.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **Standard Curve:** Reconstitute the 20 ng of Human Complement C3b Standard with 2 ml of EIA Diluent to generate a solution of 10 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 (10 ng/ml) 1:2 with EIA Diluent to produce 5, 2.5, 1.25, 0.625, 0.313, and 0.156 ng/ml solutions. EIA 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	[C3b] (ng/ml)
P1	Standard (10 ng/ml)	10.00
P2	1 part P1 + 1 part EIA Diluent	5.000
P3	1 part P2 + 1 part EIA Diluent	2.500
P4	1 part P3 + 1 part EIA Diluent	1.250
P5	1 part P4 + 1 part EIA Diluent	0.625
P6	1 part P5 + 1 part EIA Diluent	0.313
P7	1 part P6 + 1 part EIA Diluent	0.156
P8	EIA Diluent	0.000

- **Biotinylated Complement C3b Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 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

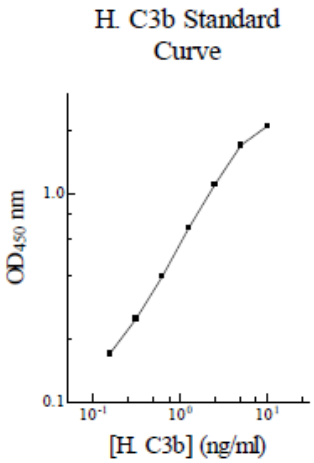
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human Complement C3b 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 C3b Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- 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 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 the 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 complement C3b is typically ~ 0.15 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1 % and 7.2 % respectively.

LINEARITY

	Average Percentage of Expected Value	
Sample Dilution	Plasma	Serum
1:200000	91%	92%
1:400000	97%	98%
1:800000	106%	108%

	Average Percentage of Expected Value
Sample Dilution	Milk
1:2000	96%
1:4000	99%
1:8000	103%

RECOVERY

Standard Added Value	0.25 – 2.5 ng/ml
Recovery %	83 - 112%
Average Recovery %	97.5%

CROSS-REACTIVITY

Species	% Cross Reactivity
Monkey	None
Mouse	None
Rat	None
Swine	None
Canine	None
Bovine	None
Human	100%
Proteins	**% Cross Reactivity**
Complement C1	None
Complement C1q	None
Complement C1r	None
Complement C1s	None
Complement C3	80%
Complement C3b	100%
Complement C4	None
Complement C5	None
Complement C6	None
Complement C7	None
Complement C8	None
Complement C9	None

REFERENCE VALUE

- Normal human C3b plasma levels range from 0.3 to 1.6 mg/ml.

REFERENCES

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(7) Stokowska A *et al.* (2011) *Cerebrovasc Dis.* 32(2):114-122

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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